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(54) Title: MULTIPLY-SUBSTITUTED PROTEASE VARIANT AND AMYLASE VARIANT-CONTAINING CLEANING COMPOSITIONS

(57) Abstract

The present invention relates to cleaning compositions comprising a protease variant. One cleaning composition comprises a protease variant including a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residue positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin; and one or more cleaning adjunct materials. Another cleaning composition comprises a protease variant including a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 27, 212, 230, 232, 252 and 257 of Bacillus amyloliquefaciens subtilisin; an amylose variant and one or more cleaning adjunct materials. Methods for using the cleaning compositions are also provided.

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# MULTIPLY-SUBSTITUTED PROTEASE VARIANT AND AMYLASE VARIANT-CONTAINING CLEANING COMPOSITIONS

### FIELD OF THE INVENTION

The present invention relates to cleaning compositions which comprise one or more protease enzymes which are multiply-substituted protease variants and one or more amylase enzymes which are amylase variants. More particularly, the present invention relates to laundry detergent compositions, dishwashing detergent compositions, hard surface cleaning compositions and personal cleansing compositions which comprise one or more multiply-substituted protease variants and one or more amylase variants.

### **BACKGROUND OF THE INVENTION**

Various types of enzymes have long been used in laundry detergents to assist in the removal of certain stains from fabrics. Each class of enzyme (amylase, protease, etc.) generally catalyzes a different chemical reaction. For example, protease enzymes are known for their ability to hydrolyze (break down a compound into two or more simpler compounds) other proteins. This ability has been taken advantage of through the incorporation of naturally occurring or engineered protease enzymes to laundry detergent compositions.

In recent years the use of enzymes has also been investigated for use in automatic dishwashing compositions. Unfortunately, many enzymes, such as many conventional protease enzymes, do not translate well into the wash environment. Specifically, thermal stability, pH stability, oxidative stability and substrate specificity need to be optimized to ensure satisfactory performance.

U.S. Patent No. RE 34,606 to Estell et al. discloses the modification of subtilisin amion acid residues corresponding to positions in *Bacillus amyloliquefaciens* subtilisin tyrosine -1, aspartate +32, asparagine +155, tyrosine +104, methionine +222, glycine +166, histidine +64, glycine +169, phenylalanine +189, serine +33, serine +221, tyrosine +217, glutamate +156 and alanine +152.

U.S. Patent No. 5,182,204 discloses the modification of the amino acid +224 residue in *Bacillus amyloliquefaciens* subtilisin and equivalent positions in other subtilisins which may be modified by way of substitution, insertion or deletion and which may be combined with modifications to the residues identified in U.S. Patent No. RE 34,606 to form useful subtilisin mutants or variants. U.S. Patent No. 5,182,204 further discloses the modification of many amino acid residues within subtilisin, including specifically +99, +101, +103, +107, +126, +128, +135, +197 and +204.

U.S. Patent No. 5,679,630 to Baeck et al. discloses cleaning compositions comprising a protease variant including substitutions of amino acid residues with other amino acid residues at positions corresponding to position 76 in combination with one or more of the following positions 99, 101, 103, 104, 107, 123, 27, 105, 109, 126, 128, 135, 156, 166, 195, 197, 204, 206, 210, 216, 217, 218, 222, 260, 265 and/or 274 of *Bacillus amyloliquefaciens* subtilisin, and one or more cleaning composition materials.

In addition to protease enzymes, amylase enzymes have been used for a variety of different purposes, the most important of which are starch liquefaction, textile desizing, starch modification in the paper and pulp industry, and for brewing and baking. A further use of amylases, which is becoming increasingly important, is the removal of starch containing soils and stains during the washing of fabrics, hard surfaces, and/or dishes.

WO 94/18314 (Genencor) published August 18, 1994, WO 94/02596 (Novo) published February 3, 1994, and WO 95/10603 (Novo) published April 20, 1995, describe cleaning compositions which incorporate mutant amylases.

Other amylases known for use in cleaning compositions include both  $\alpha$ - and  $\beta$ -amylases.  $\alpha$ -Amylases are known in the art and include those disclosed in U.S. Patent Nos. 5,003,257; EP 252 666; WO 91/00353; FR 2,676,456; EP 285 123; EP 525 610; EP 368 341; and British Patent Specification No. 1,296,839 (Novo).

WO 95/26397 (Novo) published October 5, 1995 discloses an  $\alpha$ -amylase having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10.

WO 98/05748 (P&G) published February 12, 1998 discloses variants of the  $\alpha$ -amylases described in WO 95/26397 used in detergent compositions.

WO 98/30669 (Henkel) published July 16, 1998 discloses a protease and amylase-containing detergent composition wherein the protease is a protease mutant in which the amino acid leucine present in position 211 (BLAP counting method) in the wild-type protease is exchanged at this location for an aspartic acid or glutamic acid, and the amylase is an amylae mutant in which at least one methionine, tryptophan, cysteine or tyrosine present in the wild-type amylase is removed or exchanged for another amino acid which is in particular not cysteine or methionine. Examples of amylase mutants suitable for use in

the compositions of WO 98/30669 are disclosed in WO 94/02597 (Novo), WO 95/10603 (Novo) and WO 94/18314 (Genencor) and are commercially available as Duramyl[®] (Novo) and Purafect OxAm[®] (Genencor).

However, there continues to exist a consumer need for cleaning compositions that provide more enhanced and/or improved cleaning (removal and/or reduction) of soils and/or stains from substrates over conventional enzyme-containing cleaning compositions

By the present invention, it has been found that the combination of novel protease enzymes which are multiply-substituted protease variants and amylase enzymes which are amylase variants, especially  $\alpha$ -amylase variants, provide enhanced and/or improved soil and/or stain removal benefits over conventional enzyme-containing cleaning compositions and/or over cleaning compositions containing the novel protease enzymes of the present invention in the absence of the amylase enzymes of the present invention.

Further, it has been surprisingly found that cleaning compositions comprising the novel combination of the novel protease enzymes of the present invention with the amylase enzymes of the present invention provide superior cleaning benefits over the cumulative cleaning benefits provided by cleaning compositions comprising one or the other, but not both, of the novel protease enzymes of the present invention or the amylase enzymes of the present invention.

Accordingly, it is an object of the present invention to provide cleaning compositions, especially laundry detergent compositions and/or dishwashing detergent compositions, having improved soil and/or stain removal benefits and/or fabric cleaning benefits.

Further, the specific combinations claimed in the present application are not identified in any of these prior art references.

### **SUMMARY OF THE INVENTION**

The present invention meets the aforementioned needs in that it has been surprisingly discovered that the multiply-substituted protease variants of the present invention, when used in combination with the amylase variants of the present invention in cleaning compositions provide improved and enhanced cleaning ability, including, but not limited to, stain and/or soil removal and/or reduction and/or whiteness maintenance and/or dingy cleanup and/or spot and/or film removal and/or reduction, over conventional enzyme-containing cleaning compositions.

The multiply-substituted protease variants and amylase variants of the present invention are suitable for use in high and low density granular, heavy duty and light duty liquids, tablets, as well as synthetic detergent bar compositions, and other cleaning compositions.

In one aspect of the present invention a cleaning composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is selected from the group consisting of:
- (i)  $\alpha$ -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas[®]  $\alpha$ -amylase activity assay and/or;
- (ii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv)  $\alpha$ -amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-

Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an α-amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;

- (v)  $\alpha$ -amylase according to (i-iv) wherein the  $\alpha$ -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi)  $\alpha$ -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii)  $\alpha$ -amylase showing positive immunological cross-reactivity with antibodies raised against an  $\alpha$ -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent  $\alpha$ -amylase, wherein the parent  $\alpha$ -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an α-amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an  $\alpha$ amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent α-amylase has been deleted; and/or (B) at least one amino acid residue of said parent \alpha-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α-amylase; said variant having an α-amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or  $\alpha$ -amylolytic activity at neutral to relatively high pH values; increased  $\alpha$ -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for \alpha-amylase variant to the pH of the medium; and
  - (c) one or more cleaning adjunct materials.

In yet another aspect of the present invention, a fabric cleaning composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the fabric cleaning composition of a protease variant, wherein said protease variant is as described above;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above;
- (c) at least about 5% by weight of the fabric cleaning composition of a surfactant; and
  - (d) at least about 5% by weight of the fabric cleaning composition of a builder,

is provided.

In still another aspect of the present invention, a method for cleaning a fabric in need of cleaning comprising contacting the fabric with the fabric cleaning composition of the present invention is provided.

In still yet another aspect of the present invention, a dishwashing composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the dishwashing composition of a protease variant, wherein said protease variant is as described above;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above; and
- (c) from about 0.1% to about 10% by weight of a surfactant, is provided.

In still yet another aspect of the present invention, a method for cleaning a dish in need of cleaning comprising contacting the dish with the dishwashing composition of the present invention is provided.

In still yet another aspect of the present invention, a personal cleansing composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.001% to about 5% by weight of the personal cleansing composition of a protease variant, wherein said protease variant is as described above;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above; and
- (c) from about 0.1% to about 95% by weight of the personal cleansing composition of a surfactant system; and
- (d) optionally, from about 0.05% to about 50% by weight of the personal cleansing composition of an enzyme stabilizer, is provided.

In still yet another aspect of the present invention, a method for personal cleansing of a part of the human or lower animal body in need of cleansing comprising contacting the part with the personal cleansing composition of the present invention is provided.

In still yet another aspect of the present invention, a cleaning composition comprising:

(a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of a

protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;

- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is selected from the group consisting of:
- (i)  $\alpha$ -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas®  $\alpha$ -amylase activity assay and/or;
- (ii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv) α-amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an α-amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;
- (v)  $\alpha$ -amylase according to (i-iv) wherein the  $\alpha$ -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi)  $\alpha$ -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii)  $\alpha$ -amylase showing positive immunological cross-reactivity with antibodies raised against an  $\alpha$ -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent  $\alpha$ -amylase, wherein the parent  $\alpha$ -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an  $\alpha$ -amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an  $\alpha$ -amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent  $\alpha$ -amylase has been deleted; and/or (B) at least one amino acid residue of said parent  $\alpha$ -amylase has been replaced by a different amino acid

residue; and/or (C) at least one amino acid residue has been inserted relative to said parent  $\alpha$ -amylase; said variant having an  $\alpha$ -amylase activity and exhibiting at least one of the following properties relative to said parent  $\alpha$ -amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or  $\alpha$ -amylolytic activity at neutral to relatively high pH values; increased  $\alpha$ -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for  $\alpha$ -amylase variant to the pH of the medium; and

(c) one or more cleaning adjunct materials, is provided.

In still yet another aspect of the present invention, a fabric cleaning composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the fabric cleaning composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above;
- (c) at least about 5% by weight of the fabric cleaning composition, of a surfactant; and
- (d) at least about 5% by weight of the fabric cleaning composition, of a builder, is provided.

In still another aspect of the present invention, a method for cleaning a fabric in need of cleaning comprising contacting the fabric with the fabric cleaning composition of the present invention is provided.

In still yet another aspect of the present invention, a dishwashing composition comprising:

(a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the fabric cleaning composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;

- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above; and
- (c) from about 0.1% to about 10% by weight of the dishwashing composition, of a surfactant, is provided.

In still yet another aspect of the present invention, a method for cleaning a dish in need of cleaning comprising contacting the dish with the dishwashing composition of the present invention is provided.

In still yet another aspect of the present invention, a personal cleansing composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.001% to about 5% by weight of the personal cleansing composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above; and
- (c) from about 0.1% to about 95% by weight of the personal cleansing composition, of a surfactant system; and
- (d) optionally, from about 0.05% to about 50% by weight of the personal cleansing composition, of an enzyme stabilizer, is provided.

In still yet another aspect of the present invention, a method for personal cleansing of a part of the human or lower animal body in need of cleansing comprising contacting the part with the personal cleansing composition of the present invention is provided.

Accordingly, it is an object of the present invention to provide cleaning compositions having a combination of a protease variant and amylase variant capable of providing improved and enhanced cleaning of fabrics, dishware, tableware, kitchenware, cookware and other hard surface substrates. It is a further object of the present invention to provide methods for fabric, dishware, tableware, kitchenware, cookware and other hard surface substrate cleansing via the use of the protease variant/amylase variant-containing cleaning compositions of the present invention.

These and other objects, features and advantages will be clear from the following detailed description, examples and appended claims.

All percentages, ratios and proportions herein are on a weight basis unless otherwise indicated. All documents cited herein are hereby incorporated by reference.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 A-C depict the DNA and amino acid sequence for *Bacillus* amyloliquefaciens subtilisin and a partial restriction map of this gene.

Fig. 2 depicts the conserved amino acid residues among subtilisins from *Bacillus* amyloliquefaciens (BPN)' and *Bacillus lentus* (wild-type).

Figs. 3A and 3B depict the amino acid sequence of four subtilisins. The top line represents the amino acid sequence of subtilisin from *Bacillus amyloliquefaciens* subtilisin (also sometimes referred to as subtilisin BPN'). The second line depicts the amino acid sequence of subtilisin from *Bacillus subtilis*. The third line depicts the amino acid sequence of subtilisin from *B. licheniformis*. The fourth line depicts the amino acid sequence of subtilisin from *Bacillus lentus* (also referred to as subtilisin 309 in PCT WO89/06276). The symbol * denotes the absence of specific amino acid residues as compared to subtilisin BPN'.

## **DETAILED DESCRIPTION OF THE INVENTION**

I. <u>Proteases</u> - Proteases are carbonyl hydrolases which generally act to cleave peptide bonds of proteins or peptides. As used herein, "protease" means a naturally occurring protease or recombinant protease. Naturally-occurring proteases include  $\alpha$ -aminoacylpeptide hydrolase, peptidylamino acid hydrolase, acylamino hydrolase, serine carboxypeptidase, metallocarboxypeptidase, thiol proteinase, carboxylproteinase and metalloproteinase. Serine, metallo, thiol and acid protease are included, as well as endo and exo-proteases.

The present invention includes protease enzymes which are non-naturally occurring carbonyl hydrolase variants (protease variants) having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Specifically, such protease variants have an amino acid sequence not found in nature, which is derived by replacement of a plurality of amino acid residues of a precursor protease with different amino acids. The precursor protease may be a naturally-occurring protease or recombinant protease. As stated earlier, the protease variants are designed to have trypsin-like specificity and preferably also be bleach stable.

The protease variants useful herein encompass the substitution of any of the nineteen naturally occurring L-amino acids at the designated amino acid residue positions.

Such substitutions can be made in any precursor subtilisin (procaryotic, eucaryotic, mammalian, etc.). Throughout this application reference is made to various amino acids by way of common one- and three-letter codes. Such codes are identified in Dale, M.W. (1989), Molecular Genetics of Bacteria, John Wiley & Sons, Ltd., Appendix B.

The protease variants useful herein are preferably derived from a *Bacillus* subtilisin. More preferably, the protease variants are derived from *Bacillus lentus* subtilisin and/or subtilisin 309.

<u>Carbonyl Hydrolases</u> - Carbonyl hydrolases are protease enzymes which hydrolyze compounds containing

O

II

C-X

bonds in which X is oxygen or nitrogen. They include naturally-occurring carbonyl hydrolases and recombinant carbonyl hydrolases. Naturally-occurring carbonyl hydrolases principally include hydrolases, e.g., peptide hydrolases such as subtilisins or metalloproteases. Peptide hydrolases include  $\alpha$ -aminoacylpeptide hydrolase, peptidylamino acid hydrolase, acylamino hydrolase, serine carboxypeptidase, metallocarboxypeptidase, thiol proteinase, carboxylproteinase and metalloproteinase. Serine, metallo, thiol and acid protease's are included, as well as endo and exo-proteases.

Subtilisins - Subtilisins are bacterial or fungal proteases which generally act to cleave peptide bonds of proteins or peptides. As used herein, "subtilisin" means a naturally-occurring subtilisin or a recombinant subtilisin. A series of naturally-occurring subtilisins is known to be produced and often secreted by various microbial species. Amino acid sequences of the members of this series are not entirely homologous. However, the subtilisins in this series exhibit the same or similar type of proteolytic activity. This class of serine proteases share a common amino acid sequence defining a catalytic triad which distinguishes them from the chymotrypsin related class of serine proteases. The subtilisins and chymotrypsin related serine proteases both have a catalytic triad comprising aspartate, histidine and serine. In the subtilisin related proteases the relative order of these amino acids, reading from amino to carboxy terminus, is aspartatehistidine-serine. In the chymotrypsin related proteases, the relative order, however, is histidine-aspartate-serine. Thus, subtilisin herein refers to a serine protease having the catalytic triad of subtilisin related proteases. Examples include, but are not limited to, the subtilisins identified in Fig. 3 herein. Generally, and for purposes of the present invention, numbering of the amino acids in proteases corresponds to the numbers assigned to the mature Bacillus amyloliquefaciens subtilisin sequence presented in Fig. 1.

Protease Variants - A "protease variant" has an amino acid sequence which is derived from the amino acid sequence of a "precursor protease." The precursor proteases include naturally-occurring proteases and recombinant proteases. The amino acid sequence of the protease variant is "derived" from the precursor protease amino acid sequence by substitution, deletion or insertion of one or more amino acids of the precursor amino acid sequence. Such modification is of the "precursor DNA sequence" which encodes the amino acid sequence of the precursor protease rather than manipulation of the precursor protease enzyme per se. Suitable methods for such manipulation of the precursor DNA sequence include methods disclosed herein, as well as methods know to those skilled in the art (see, for example, EP 0 328 299, WO 89/06279 and the U.S. patents and applications already referenced herein).

In a preferred embodiment, the protease variants which are protease enzymes useful in the present invention cleaning compositions comprise protease variants including a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin; and one or more cleaning adjunct materials.

While any combination of the above listed amino acid substitutions may be employed, the preferred protease variant enzymes useful for the present invention comprise the substitution, deletion or insertion of amino acid residues in the following combinations:

(1) a protease variant including substitutions of the amino acid residues at position 103 and at one or more of the following positions 236 and 245;

- (2) a protease variant including substitutions of the amino acid residues at positions 103 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 248, 252, 257, 260, 270 and 275;
- (3) a protease variant including substitutions of the amino acid residues at positions 103 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 170, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 222, 230, 232, 248, 252, 257, 260, 261, 270 and 275; and
- (4) a protease variant including substitutions of the amino acid residues at positions 103, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 243, 248, 252, 257, 260, 270 and 275.

A more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table I) selected from the group consisting of:

Table I

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76	103	104	160					<u> </u>				
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19	76	103	104	-	<del> </del>	_	+-					<del> </del>
76	103	104	184	+	+-	_				+	<b>_</b>	
76	103	104	252	-	<del> </del>						-	
76	103	104	259	<del> </del>	+		-					
76	103	104	251	<del> </del>	<del> </del>	+	+					
76	86	103	104	1	-		+	-		<u> </u>		
72	76	103	104	185	-	<del></del>					<del> </del>	
76	103	104	237	274		+		+		-		
76	103	104	160	<del>                                     </del>				<del> </del>			<del> </del> -	
76	103	104	228			<del>                                     </del>	+	<del></del> -				
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76	103	104	254				+-					
76	103	104	204				+			<del> </del>	+	
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76	103	104	159			1		+		<del> </del>		
10	76	103	104	177			1	-	+	+		
58	76	103	104				<del>                                     </del>	<del> </del>	-	<del> </del>		
76	103	104	270				<del>                                     </del>	+	┼	+		
76	103	104	185				-	<del> </del>	+	<del> </del>		
27	76	103	104		<del></del>		†	+-	<del> </del>	<del>                                     </del>		
76	103	104	262				†	+		-		
76	78	103	104			1	<del> </del>	+	<del> </del>	<del> </del>		
24	76	103	104		<del></del>		<del>                                     </del>	+	<del>                                     </del>	-		
76	103	104	166	236	251			_	+	-		
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76	103	104	137	238	271	<b>†</b>		1	$\dashv$			+	<del> </del>	$\dashv$
103	104	228						-	+			<u> </u>		$\dashv$
76	103	104	182	198		<del>                                     </del>		+	$\dashv$				<del> </del>	$\dashv$
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76	103	104	137	248				-	$\top$			<del>                                     </del>		$\dashv$
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58	76	103	104	271				<del> </del>	+	$\dashv$	·	<del> </del>	<del> </del>	+
76	103	104	206	261				<del>                                     </del>	+			<del>                                     </del>		$\dashv$
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68	76	103	104	159	236	_	+	_				<del> </del>	<del> </del>
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76	103	104	146	159			<del> </del>	+					
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76	103	104	159	224	<b></b> -		<del> </del>	+		-			
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76	87	103	104	212	271		†	+	-				
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76	103	104	212	236	243	271	<u> </u>	+-	_				
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68	76	103	104	159	236	271		+-	+				
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68	76	103	104	159	209	236	253	-		-	<u> </u>	
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68	76	103	104	159	236	245	247					
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68	103	104	159	215	232	236	245		<del> </del>			
68	103	104	159	232	236	245	248	1	<del>                                     </del>	1	<u> </u>	
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68	76	103	104	159	210	232	236	245			<del> </del>	
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68	103	104	159	232	236	245	257	275		†		<del></del>
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68	103	104	159	224	232	236	245	257			<del> </del>	<del>                                     </del>
76	103	104	159	232	236	245	257	<u> </u>				
68	76	103	104	159	209	232	236	245				
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12	68	76	103	104	159	214	232	236	245	<del>                                     </del>		
68	76	103	104	159	215	232	236	245		<del> </del> -		
12	68	76	103	104	159	232	236	245				
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68	87	76	103	104	159	232	236	245	260		<del> </del>	
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76	103	104	159	192	232	236	245					
76	103	104	147	159	232	236	245	248	251			
12	68	76	103	104	159	232	236	245	272			
68	76	103	104	159	183	206	232	236	245			
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10	3 104	1 159	9 232	2 236	245	248	252	-		+	+-		
68	103	104	1 159	209	232	236	245	248	252	+	+		
68	103	104	1 109	159	232	236	245	248	252	<del></del>	-		
20	68	103	104	159	232	236	245	248	252	<del></del>			
68	103	104	159	209	232	236	245	248	252	+	+		
68	103	104	159	232	236	245	248	252	261	+	-		
68	103	104	159	185	232	236	245	248	252	<del> </del>	<del> </del>		
68	103	104	159	210	232	236	245	248	252	<del> </del> -	┼	-	
68	103	104	159	185	210	232	236	245	248	252	-		
68	103	104	159	212	232	236	245	248	252	<del> </del>			
68	103	104	159	213	232	236	245	248	252	<del> </del>	-		
68	103	104	213	232	236	245	248	252				+	
68	103	104	159	215	232	236	245	248	252	<del> </del>	-	+	
68	103	104	159	216	232	236	245	248	252			+	{
20	68	103	104	159	232	236	245	248	252			+	
68	103	104	159	173	232	236	245	248	252			+	
68	103	104	159	232	236	245	248	251	252			<del></del>	
68	103	104	159	206	232	236	245	248	252			<del></del>	
68	103	104	159	232	236	245	248	252				+	$\dashv$
55	68	103	104	159	232	236	245	248	252			+	
68	103	104	159	232	236	245	248	252	255			<del> </del>	$\dashv$
68	103	104	159	. 232	236	245	248	252	256				$\dashv$
68	103	104	159	232	236	245	248	252	260			-	$\dashv$
68	103	104	159	232	236	245	248	252	257			<del> </del>	_
68	103	104	159	232	236	245	248	252	258			<del> </del>	
8	68	103	104	159	232	236	245	248	252	269		<del> </del>	_
			L	L				L					

68	103	104	116	159	232	236	245	248	252	260		
68	103	104	159	232	236	245	248	252	261			
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68	103	104	159	232	236	245	248	252				
68	76	101	103	104	159	213	218	232	236	245	260	
68	103	104	159	228	232	236	245	248	252			
33	68	76	103	104	159	232	236	245	248	252		
68	76	89	103	104	159	210	213	232	236	245	260	
61	68	76	103	104	159	232	236	245	248	252		
103	104	159	205	210	232	236	245					
61	68	103	104	130	159	232	236	245	248	252		
61	68	103	104	133	137	159	232	236	245	248	252	
61	103	104	133	159	232	236	245	248	252			
68	103	104	159	232	236	245	248	252				
68	103	104	159	218	232	236	245	248	252			
61	68	103	104	159	160	232	236	245	248	252		
3	61	68	76	103	104	232	236	245	248	252		
61	68	103	104	159	167	232	236	245	248	252		
97	103	104	159	232	236	245	248	252				
98	103	104	159	232	236	245	248	252	-			
99	103	104	159	232	236	245	248	252				
101	103	104	159	232	236	245	248	252				
102	103	104	159	232	236	245	248	252				
103	104	106	159	232	236	245	248	252				
103	104	109	159	232	236	245	248	252				
103	104	159	232	236	245	248	252	261				
62	103	104	159	232	236	245	248	252				

10	3   104	159	184	232	236	245	248	252			<b>T</b>	1
10	3 104	159	166	232	236	245	248	252	+	<del>                                     </del>	+	
10:	3 104	159	217	232	236	245	248	252	<del> </del>	-	+	<del></del>
20	62	103	104	159	213	232	236	245	248	252	-	+
62	103	104	159	213	232	236	245	248	252	†	-	<del> </del>
103	3 104	159	206	217	232	236	245	248	252	-		<del> </del>
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38	76	103	104	159	213	232	236	245	260			
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68	76	103	104	159	209	213	232	236	245	260		
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68	76	103	104	159	210	232	236	245	260			
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76	103	104	159	213	232	236	245	260				
68	103	104	159	209	232	236	245					
68	103	104	159	210	232	236	245					
68	103	104	159	230	232	236	245					
68	103	104	159	126	232	236	245					
68	103	104	159	205	232	236	245					
68	103	104	159	210	232	236	245					
103	104	159	230	236	245							
68	103	104	159	232	236	245	260				<del>-  </del>	
103	104	159	232	236	245							
68	103	104	159	174	232	236	245	257				
68	103	104	159	194	232	236	245	257				
68	103	104	159	209	232	236	245	257				
103	104	159	232	236	245	257			-+			

68	76	103	104	159	213	232	236	245	260	261		
68	103	104	159	232	236	245	257	261				
103	104	159	213	232	236	245	260	!	1	†	1 -	†
103	104	159	210	232	236	245	248	252		†	<b>†</b>	<del>                                     </del>
103	104	159	209	232	236	245	257			†	<del> </del>	
68	76	103	104	159	210	213	232	236	245	260		<del> </del>
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103	104	209	232	236	245	257				<b>†</b>	<del>                                     </del>	<del>                                     </del>
103	104	159	205	210	213	232	236	245	260	<del> </del>		<del> </del>
103	104	159	205	209	232	236	245	260				
68	103	104	159	205	209	210	232	236	245		<del>                                     </del>	<del> </del>
103	104	159	205	209	210	232	236	245	257			
103	104	159	205	209	232	236	245	257				
68	103	104	159	205	209	210	232	236	245	260		
103	104	159	205	209	210	232	236	245				
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103	104	159	205	210	232	236	245					
68	103	104	128	159	232	236	245					<del> </del>
48	103	104	159	230	236	245						
48	68	103	104	159	209	232	236	245				<u> </u>
48	68	103	104	159	232	236	245	248	252			<u> </u>
48	68	103	104	159	232	236	245	257	261			
102	103	104	159	212	232	236	245	248	252			
12	102	103	104	159	212	232	236	245	248	252		
101	102	103	104	159	212	232	236	245	248	252		
98	102	103	104	159	212	232	236	245	248	252		
102	103	104	159	213	232	236	245	248	252			
103	104	131	159	232	236	245	248	252				
103	104	159	184	232	236	245	248	252				
103	104	159	232	236	244	245	248	252			<del></del>	
62	103	104	159	213	232	236	245	248	252	256		
12	62	103	104	159	213	232	236	245	248	252		
										L		

10	1 103	3 104	1 159	185	232	236	245	248	252		1	<del></del>
10	1 103	3 104	1 159	206	232	236	245	248	252	+	+	
10	1 103	104	159	213	232	236	245	248	252	+	<del></del> -	
98	102	103	104	159	232	236	245	248	252	+-	<del> </del>	<del></del>
10	1 102	103	104	159	232	236	245	248	252	-	-	
98	102	103	104	159	212	232	236	245	248	252	-	
98	102	103	104	159	212	232	236	248	252	+-	+	<del> </del>
62	103	104	109	159	213	232	236	245	248	252	+	<del></del> -
62		104	159	212	213	232	236	245	248	252	<del>-</del>	
62	101	103	104	159	212	213	232	236	245	248	252	
103		159	232	245	248	252			<del>                                     </del>		<del> </del>	
103		159	230	245					<del>                                     </del>	<del>                                     </del>		<del> </del>
62	103	104	130	159	213	232	236	245	248	252	1	
101		104	130	159	232	236	245	248	252	<del>                                     </del>		
101	<u> </u>	104	128	159	232	236	245	248	252			
62	101	103	104	159	213	232	236	245	248	252	<del>                                     </del>	
62	103	104	128	159	213	232	236	245	248	252	<del>                                     </del>	<del> </del>
62	103	104	128	159	213	232	236	245	248	252	1	<del>                                     </del>
101	103	104	159	232	236	245	248	252	260			
101	103	104	131	159	232	236	245	248	252			
98	101	103	104	159	232	236	245	248	252			
99	101	103	104	159	232	236	245	248	252			
101	103	104	159	212	232	236	245	248	252			
76	103	104	167	170	194							
101	103	104	159	209	232	236	245	248	252			
101	103	104	159	210	232	236	245	248	252			
101	103	104	159	205	232	236	245	248	252			
101	103	104	159	230	236	245						
101	103	104	159	194	232	236	245	248	252			
76	101	103	104	159	194	232	236	245	248	252		
101	103	104	159	230	232	236	245	248	252			
62	103	104	159	185	206	213	232	236	245	248	252	271

An even more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table II) selected from the group consisting of:

Table II

1		T				140101					
<u> </u>		-									
N76	D A98E	S103/	N V104	1						7	7
N76	D S78T	S103A	V104	1							<del></del>
N76	D S103	V104	I 1107\	/							+
V4E	N76D	S103A	V104	1							
N76	D S103A	V104	I246V	,							
N761	D N77D	S103A	V104	1						<del> </del>	-
N761	S103A	V1041	N1831	N218I							
A16	Г N76D	S103A	V1041	N248D			1	T	<del>                                     </del>	<del>                                     </del>	
AlE	N76D	S103A	V104I						<del>                                     </del>	†	+
N76I	S103A	V104I	N261E				1		<b>-</b>		
N76I	S103A	V1041	S160T							1	<del> </del>
N76I	S103A	V1041	S216C							+	<del>                                     </del>
H170	N76D	S103A	V104I							†	<del> </del>
S37T	N76D	S103A	V1041						1		<del>                                     </del>
N76E	N77D	S103A	V104I	A174V						<del> </del>	
T38S	N76D	S103A	V104I								
T38S	N76D	S103A	V104I	K237Q							
I8V	N76D	S103A	V1041					1	<b>†</b>		
N76D	S103A	V104I	N183D						<u> </u>		
R19L	N76D	S103A	V104I						1		
A13V	N76D	S103A	V104I								
R19C	N76D	S103A	V104I						<b>†</b>		
N76D	S103A	V104I	N184D								
N76D	S103A	V104I	N252D						<del> </del>		
N76D	S103A	V104I	S259C					<u> </u>			
N76D	S103A	V104I	K251T								
N76D	P86S	S103A	V104I								
172V	N76D	S103A	V104I	N185D	<del></del>		 <del>                                     </del>		<del> </del>		
N76D	S103A	V104I	K237E	T274A					<del>                                     </del>		
N76D	S103A	V104I	S160L					<del>                                     </del>			

S103	A V104	I G159	D A2321	/ Q236I	1 V244	A Q2451	R N248	D N252	К		T	<del></del>
N62	D S103	A V104	I G159I	ŧ .					D N252I	S256F	,	+
Q121	R N621	S103/	A V1041	1					R N248I			
S101	G S103/	V104	I G159E	N185E		1	ł	1	D N2521		<del>`</del>	
S101	G S103	V104	I G159E	Q206E	A232V				N2521	<del></del>	<del> </del>	
S101	G S103A	V104	G159E	T213Q	A232V				N252k	<del></del>	<del> </del>	+
A981	_ G102/	S103A	V1041	G159E	A232V				N252K		<del> </del>	+
S1010	G G102/	S103A	V1041			1	1		N252K	<del></del>	<del> </del>	+
A981	G102A	S103A	V1041		1	1			N248E	<del></del>		-
A981	. G102A	S103A	V1041	G159D	1	1	1	,	N252K		<del>`</del>	-
N62I	S103A	V1041	Q109R	G159D		1			N248D			<del>-</del>
N62E	S103A	V1041	G159D	S212G		i			N248D		<del>                                     </del>	<del></del> -
N62E	S101G	S103A	V104I	G159D	S212G	T213R	<del></del>		Q245R	<del>                                     </del>	<del> </del>	-
S103A	V104I	G159D	A232V	Q245R	N248D	T	1		1	1.2,00	1.12321	+
S103A	V1041	G159D	A230V	Q245R					<del> </del> -	<del>                                     </del>		+
N62D	S103A	V1041	S130G	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K	<del>                                     </del>	<del>                                     </del>
S101C	S103A	V104I	S130G	1	1	4	1		N252K			+
S101C	S103A	V104I	S128G	i		1	1		N252K	<del></del>		<del> </del>
S101G	S103A	V104I	S128L	ľ	l .	1			N252K	+		<del> </del>
N62D	S101G	S103A	V104I	G159D		1			N248D			
N62D	S103A	V104I	S128G	G159D	1	i			N248D			
N62D	S103A	V1041	S128L	G159D					N248D			<del>                                     </del>
S101G	S103A	V1041	G159D	A232V					T260A			<del>                                     </del>
S101G	S103A	V1041	P131V						N252K			
A98V	S101G	S103A										
S99G	S101G	S103A		G159D		1						
S101G	S103A	V104I		S212G	1							
S101G	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	G159D		1	- 1		N248D			·	
S101G	S103A	V104I	G159D	V2051		Q236H			N252K			
S101G	S103A	V104I	G159D	A230V		Q245R						
S101G	S103A	V104I	G159D			<del></del>	Q245R	N248D	N252K		· · · · · · · · · · · · · · · · · · ·	
N76D	S101G	S103A	V1041	1	- 1				N248D	N252K		
S101G	S103A	V104I	G159D					N248D		1232K		
N62D	S103A		G159D 1			T213R			Q245R	NOVED	אומגמע	E2710
					<u> </u>			V23011	Q243K	N248D	N232K	E2/1Q

N76D   S103A   V1041   Q182R   1198V		Τ		<del>                                     </del>	T	1	τ	<del></del>	Т	 		
L21M	S103A	V104I	A228T	ļ					ļ <u>.</u> .			
N76D   S103A   V104I   M119I   Q137R   N76D   S103A   V104I   Q206R   N76D   S103A   V104I   G150D   C206E   N76D   S103A   V104I   G150D   C206E   N76D   S103A   V104I   C206E   N76D   S103A   V104I   Q206E   N76D   S103A   V104I   G159D   L217E   K251Q   N76D   S103A   V104I   G159D   L217E   K251D   N76D   N77D   S103A   V104I   G159D   L217E   N252D   N76D   N77D   S103A   V104I   G159D   L217E   N252D   N76D   S103A   V104I   G159D   Q206E   V244A   N76D   S103A   V104I   S188E   N76D   S103A   V104I   S188E   N76D   N77D   S103A   V104I   S188E   N76D   N77D   S103A   V104I   S188E   N76D   N77D   S103A   V104I   N185D   N76D   N77D   S103A   V104I   N185D   N76D   S103A   V104I   Q206E   K251T   N76D   S103A   V104I   Q206E   K251T   N76D   S103A   V104I   G159D   Q236H   M24V   N76D   S103A   V104I   G159D   Q236H   M24V   N76D   S103A   V104I   G159D   Q236H   M24V   N76D   S103A   V104I   G159D   M238S   M36D   M36D	N76D	S103A	V104I	Q182R	I198V				ļ			
N76D   S103A   V104I   Q137R   N248S	L21M	N76D	S103A	V104I	Q182R							
A13T	N76D	S103A	V104I	M119I	Q137R							
N76D   S103A   V1041   Q206R	N76D	S103A	V1041	Q137R	N248S							
N76D   S103A   V1041   S212P   G258R	A13T	N76D	S103A	V104I	Q206R							
T58S         N76D         S103A         V104I         E271G            N76D         S103A         V104I         Q206E             V4E         N76D         S103A         V104I         Q206E             N76D         N77D         S103A         V104I         Q206E             N76D         S103A         V104I         Q206E              N76D         S103A         V104I         G159D         L217E         K251Q             V4E         N76D         S103A         V104I         G159D         L217E         K252D              N76D         N77D         S103A         V104I         G159D         L217E         K251D	N76D	S103A	V1041	Q206R								
N76D         S103A         V104I         Q206E         N26ID            V4E         N76D         S103A         V104I         Q206E             N76D         N77D         S103A         V104I         Q206E             N76D         S103A         V104I         Q206E              V4E         N76D         S103A         V104I         G159D         L217E         K251Q            V4E         N76D         S103A         V104I         G159D         L217E         K251Q            V4E         N76D         S103A         V104I         G159D         L217E         N252D             N76D         N77D         S103A         V104I         G159D         K251T	N76D	S103A	V1041	S212P	G258R							
V4E         N76D         S103A         V104I         Q206E           N76D         N77D         S103A         V104I         Q206E           N76D         S103A         V104I         A158E            N76D         S103A         V104I         Q206E            V4E         N76D         S103A         V104I         G159D         L217E         K251Q           V4E         N76D         S103A         V104I         G159D         L217E         N252D           N76D         N77D         S103A         V104I         A133T         N185D         K251T           N76D         S103A         V104I         G159D         Q206E         V244A            V4E         N76D         S103A         V104I         S188E             V4E         N76D         S103A         V104I         N185D             N76D         S103A         V104I         N185D              N76D         S103A         V104I         N185D              N76D         S103A         V104I         G159D <td>T58S</td> <td>N76D</td> <td>S103A</td> <td>V104I</td> <td>E271G</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	T58S	N76D	S103A	V104I	E271G							
N76D       N77D       \$103A       V1041       Q206E	N76D	S103A	V104I	Q206E	N261D							
N76D       S103A       V104I       A158E	V4E	N76D	S103A	V104I	Q206E							
N76D         S103A         V1041         Q206E           V4E         N76D         S103A         V1041         G159D         L217E         K251Q           V4E         N76D         S103A         V1041         G159D         L217E         N252D           N76D         N77D         S103A         V1041         A133T         N185D         K251T           N76D         S103A         V1041         G159D         Q206E         V244A           V4E         N76D         S103A         V1041         S188E	N76D	N77D	S103A	V1041	Q206E							
V4E         N76D         S103A         V1041         G159D         L217E         K251Q           V4E         N76D         S103A         V1041         G159D         L217E         N252D           N76D         N77D         S103A         V1041         A133T         N185D         K251T           N76D         S103A         V1041         G159D         Q206E         V244A           V4E         N76D         S103A         V1041         S188E	N76D	S103A	V104I	A158E								
V4E         N76D         S103A         V1041         G159D         L217E         N252D           N76D         N77D         S103A         V1041         A133T         N185D         K251T           N76D         S103A         V1041         G159D         Q206E         V244A         V244A           V4E         N76D         S103A         V1041         S188E         V244A         V244A           N76D         S103A         V1041         A158E         V244A         V2	N76D	S103A	V1041	Q206E								
N76D         N77D         S103A         V104I         A133T         N185D         K251T           N76D         S103A         V104I         G159D         Q206E         V244A	V4E	N76D	S103A	V1041	G159D	L217E	K251Q					
N76D       S103A       V1041       G159D       Q206E       V244A          V4E       N76D       S103A       V104I       S188E           V4E       N76D       S103A       V104I       A158E           N76D       N77D       S103A       V104I       N185D            N76D       S103A       V104I       Q206E       K251T                                                                         <	V4E	N76D	S103A	V1041	G159D	L217E	N252D					
V4E       N76D       S103A       V1041       S188E         V4E       N76D       S103A       V1041       A158E         N76D       N77D       S103A       V1041       N185D         N76D       S103A       V1041       Q206E       K251T         A48T       N76D       S103A       V1041       L111M       G159D         V68A       N76D       S103A       V1041       G159D       O236H         L42V       N76D       S103A       V1041       G159D       O12H         Q12H       N62H       N76D       S103A       V1041       G159D       O12H         N76D       S103A       V1041       G159D       O124A       O124A       O124A	N76D	N77D	S103A	V1041	A133T	N185D	K251T				-	
V4E       N76D       S103A       V104I       A158E         N76D       N77D       S103A       V104I       N185D         N76D       S103A       V104I       Q206E       K251T         A48T       N76D       S103A       V104I       L111M       G159D         V68A       N76D       S103A       V104I       G159D       Q236H         L42V       N76D       S103A       V104I       G159D       Q12H         Q12H       N62H       N76D       S103A       V104I       G159D       Q159D         L42I       N76D       S103A       V104I       G159D       Q159D       Q159D         N76D       S103A       V104I       G159D       N238S       Q159D       Q159D         N76D       S103A       V104I       G159D       T224A       Q159D       Q159D       Q159D	N76D	S103A	V1041	G159D	Q206E	V244A						
N76D       N77D       S103A       V104I       N185D         N76D       S103A       V104I       Q206E       K251T         A48T       N76D       S103A       V104I       L111M       G159D         V68A       N76D       S103A       V104I       G159D	V4E	N76D	S103A	V1041	S188E						_	
N76D       S103A       V104I       Q206E       K251T          A48T       N76D       S103A       V104I       L111M       G159D          V68A       N76D       S103A       V104I       G159D           L42V       N76D       S103A       V104I       G159D           Q12H       N62H       N76D       S103A       V104I       G159D           L42I       N76D       S103A       V104I       G159D            N76D       S103A       V104I       G159D       N238S           N76D       S103A       V104I       G159D       T224A	V4E	N76D	S103A	V1041	A158E							
A48T       N76D       S103A       V104I       L111M       G159D         V68A       N76D       S103A       V104I       G159D          L42V       N76D       S103A       V104I       G159D          Q12H       N62H       N76D       S103A       V104I       G159D          L42I       N76D       S103A       V104I       G159D           N76D       S103A       V104I       G159D       N238S           N76D       S103A       V104I       G159D       T224A	N76D	N77D	S103A	V1041	N185D							
V68A       N76D       S103A       V104I       G159D       Q236H         L42V       N76D       S103A       V104I       G159D	N76D	S103A	V1041	Q206E	K251T							
L42V       N76D       S103A       V104I       G159D         Q12H       N62H       N76D       S103A       V104I       G159D         L42I       N76D       S103A       V104I       G159D         N76D       S103A       V104I       G159D       N238S         N76D       S103A       V104I       G159D       T224A	A48T	N76D	S103A	V1041	LIIIM	G159D					·	
Q12H       N62H       N76D       S103A       V104I       G159D         L42I       N76D       S103A       V104I       G159D          N76D       S103A       V104I       G146S       G159D          N76D       S103A       V104I       G159D       N238S          N76D       S103A       V104I       G159D       T224A	V68A	N76D	S103A	V1041	G159D	Q236H						
L421       N76D       S103A       V104I       G159D          N76D       S103A       V104I       G146S       G159D          N76D       S103A       V104I       G159D       N238S          N76D       S103A       V104I       G159D       T224A	L42V	N76D	S103A	V1041	G159D					-		
N76D       S103A       V104I       G146S       G159D         N76D       S103A       V104I       G159D       N238S         N76D       S103A       V104I       G159D       T224A	Q12H	N62H	N76D	S103A	V104I	G159D						
N76D         S103A         V104I         G159D         N238S           N76D         S103A         V104I         G159D         T224A	L421	N76D	S103A	V1041	G159D							
N76D S103A V1041 G159D T224A	N76D	S103A	V104I	G146S	G159D							
	N76D	S103A	V104I	G159D	N238S							
	N76D	S103A	V104I	G159D	T224A							
N76D   S103A   V104I   S212P   V268F   E271V	N76D	S103A	V104I	S212P	V268F	E271V						
N76D E89A S103A V104I	N76D	E89A	S103A	V104I								
N76D S87R S103A V104I S212P E271V	N76D	S87R	S103A	V104I	S212P	E271V						
N76D S103A V104I S212P Q245L E271V	N76D	S103A	V1041	S212P	Q245L	E271V						
N76D S103A V104I T134S S141N S212P E271V	N76D	S103A	V104I	T134S	S141N	S212P	E271V				-	
NICE CHOOK WAS SOUD COOK	N76D	S103A	V104I	S212P	Q236L	N243S	E271V					

N76I	S103/	A V104	I Q1091	R Q245F									
N76I	S103A	V104	I Q1091	R P210L	,							_	
G201	/ N62S	N76E	S103	V104I							_		
V68A	N76D	S103A	V104	Q236H	ı						_	$\dashv$	
V68A	N76D	S103A	V1041	G159D	Q236I	I E271V	/						
V68A	N76D	S103A	V1041	G159D	Q236F	1 Q2451	2				_	$\neg +$	
V68A	N76D	S103A	V1041	G159D	L2171	Q236I	1 E271	/				$\neg +$	
H17Q	V68A	N76D	S103A	V104I								_	
V68A	N76D	S103A	V1041										
V68A	N76D	S103A	V104I	G159D	Q236R								
V68A	L75R	N76D	S103A	V104I	G159E	Q2361	1						
V68A	N76D	N76D	S103A	A114V	V1211	G159E	Q236I	I Q245I	2				
Q12R	V68A	N76D	S103A	V1041	G159D	Q236F	ı					$\neg +$	
V68A	N76D	S103A	V104I	G159D	Y209S	Q236H	T253K			<u> </u>		$\neg$	
V68A	N76D	S103A	V104I	NI17K	G159D	N184S	Q236I						
V68A	N76D	S103A	V1041	G159D	Q236H	N2431							
V68A	N76D	S103A	V104I	G159D	Q236H	Q245L							
V68A	N76D	S103A	V1041	A142V	G159D								
V68A	N76D	S103A	V1041	N123S	G159D	Q236H	H249Y					7	
V68A	N76D	S103A	V104I	G159D	Q236H	H249Q							
N76D	S103A	V1041	M222S	Q245R			<u></u>						
Q12R	N76D	S103A	V104I	M222S	H249R								
N76D	S103A	V104I	N173R	M222S									
N76D	S103A	V104I	M222S										
L21M	N76D	S103A	V1041	M222S	K237R	Y263F							
N76D	S103A	V104I	Q109R	M222S									
N76D	S103A	V104I	Q109R	M222S	E271D								
G61R	N76D	S103A	V104I	M222S									
N76D	S103A	V104I	Q137R	M222S									
N76D	S103A	V104I	Q109R	M222S	N248S	_							
N76D	S103A	V104I	M222S	H249R									
V68A	N76D	S103A	V1041	G159D	Q236H	Q245R	N261D						
V68A	N76D	S103A	V104I	S141N	G159D	Q236H	Q245R	T255S					
V68A	N76D	S103A	V1041	G159D	Q236H	Q245R	R247H					$\top$	
V68A	N76D	S103A	V104I	G159D	A174V	N204D	Q236H	Q245R					
V68A	N76D	S103A	V104I	G159D 1	V204D	Q236H	Q245R						
								_					

			<del></del>							 	
V68A	N76D	S103A	V104I	A133V	G159D	N218D	Q236H	Q245R			
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R				
V68A	N76D	S103A	V104I	G159D	A 1941	V203A	Q236H	Q245R			
Q12R	N76D	S103A	V1041	M222S	Q245R						
N76D	S103A	V104I	A232V	Q245R	<u> </u>						
S24T	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R			
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N252K				
V68A	N76D	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	T260A		
Q12R	N76D	S103A	1104T	M222S	V244I	Q245R					
Q12R	N76D	S103A	M222S	P210T	Q245R						
Q12R	N76D	S103A	1104T	S130T	M222S	Q245R					
T22K	V68A	N76D	S103A	V104I							
V68A	N76D	S103A	V1041	N184D							
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R					
V68A	S103A	V104I	N140D	G159D	A232V	Q236H	Q245R	N252K			
N43S	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N252K			
N43K	V68A	S103A	V1041	G159D	A232V	Q236H	Q245R				
N43D	V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N252K			
V68A	S87G	S103A	V1041	G159D	A232V	Q236H	Q245R	N252K	R275S		
Q12R	N76D	S103A	1104T	S130T	M222S	Q245R	N248S	L262M			
Q12R	N76D	S103A	I104T	S130T	A215V	M222S	Q245R				
Q12R	N76D	S103A	1104T	S130T	M222S	V227A	Q245R	L262S			
Q12R	N76D	S103A	1104T	S130T	A215T	M222S	Q245R				
Q12R	N76D	S103A	1104T	S130T	M222S	Q245R	N261D				
N76D	S103A	1104T	S130T	M222S	Q245R						
Q12R	N76D	S103A	1104T	S130T	N218D	M222S	Q245R	L262S	N269D		
Q12R	S57P	N76D	S103A	I104T	S130T	M222S	Q245R	K251Q			
Q12R	N76D	S103A	I104T	S130T	R170S	N185D	M222S	N243D	Q245R		
Q12R	N76D	S103A	I104T	S130T	M222S	Q245R	V268A				
Q12R	N76D	S103A	I104T	S130T	M222S	P210S	Q245R				
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V				
V68A	S103A	V104I	N116D	G159D	A232V	Q236H	Q245R				
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D				
R10C	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	V203E	A232V	Q236H	Q245R				

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V68.	A S103	A V104	II G159	D A232	V Q236	H K237	E Q245	R					
V68.	A N761	) 179N	I S103	A V104	I G159	D A232		H Q245	R				
V68/	A S103	4 V104	I G159	D N1831	D A232	V Q2361					_		
V68/	A S103	A V104	I G1591	D A174		L A232		H Q245	R			+	
V68/	A S103/	V104	I G1591	S1880	A232	V Q2361					_	+	
V68/	S103A	V 104	I G1591	A2307		V Q236I						-	
V68/	A 98T	S103A	A V104	G159I	A232V	/ Q236I	1 Q2451	R				_	
V68A	S103A	V104	I G159I	A2157	A232V	/ Q236I	H Q2451	2				-	
V68A	S103A	V104	I G159I	A2321	/ Q236H	H Q245F	N248	S				-	
V68A	N76D	S103A	V1041	G159E	A232V	/ Q236H	1 Q245F	2					
V68A	N76D	S103A	V1041	G159E	P210R	A232\	/ Q2361	1 Q2451	₹			1	
V68A	N76D	S103A	V104I	G159E	A232V	/ Q236H	I Q245F	L257	/		+	_	_
N76D	S103A	V1041	A232V	Q236H	Q245R						1		
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	L257V	R275H	1	1			_
N76D	S103A	V1041	L257V	R275H								+	
V68A	S103A	V104I	G159D	T224A	A232V	Q236H	Q245R	L257V	,		-		
N76D	S103A	V1041	G159D	A232V	Q236H	Q245R	L257V					1	
V68A	N76D	S103A	V1041	G159D	Y209W	A232V	Q236H	Q245R				+	
V68A	N76D	S103A	V104I	G159D	G211R	A232V	Q236H	Q245R			1	1	
V68A	N76D	S103A	V1041	G159D	G211V	A232V	Q236H	Q245R				1	$\exists$
Q12R	V68A	N76D	S103A	V1041	G159D	Y214L	A232V	Q236H	Q245R		1		٦
V68A	N76D	S103A	V1041	G159D	A215R	A232V	Q236H	Q245R				1	
Q12R	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R					٦
G20R	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R	S259G			1	٦
V68A	S87R	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R	T260V				
V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R	N261G					٦
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N261W					
N76D	S103A	V1041	A232V	Q236H	S242P	Q245R							7
V68A	N76D	S103A	V104I	G159D	P210L	A232V	Q236H	Q245R					
Q12R	A48V	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R				
N76D	S103A	V104I	A232V	Q236H	Q245R								
N76D	S103A	V1041	G159D	Y192F	A232V	Q236H	Q245R						$\exists$
N76D	S103A	V1041	V1471	G159D	A232V	Q236H	Q245R	N248S	K251R				
Q12R	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	A272S				
V68A	N76D	S103A	V1041	G159D	N183K	Q206L	A232V	Q236H	Q245R				$\exists$
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	S256R					$\exists$
							-						

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V68A	N76D	S103A	V1041	G159D	Q206R	A232V	Q236H	Q245R				
K27R	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R				
V68A	N76D	S103A	V104I	N116T	G159D	R170S	N185S	A232V	Q236H	Q245R		
G61E	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
N43D	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	S212P	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	S99N	G159D	N184D	A232V	Q236H	Q245R	N248D	N252K		
S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K					
V68A	S103A	V1041	G159D	Y209W	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	Q109R	G159D	A232V	Q236H	Q245R	N248D	N252K			
G20R	V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	Y209F	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K	N261D			
V68A	S103A	V104I	G159D	N185D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	P210R	A232V	Q236H	Q245R	N248D	N252K		<del>''                                   </del>	
V68A	S103A	V1041	G159D	P210T	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	P210S	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	N185D	P210L	A232V	Q236H	Q245R	N248D	N252K		
V68A	S103A	V1041	G159D	P210L	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212A	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	S212E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	T213E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	T213S	A232V	Q236H	Q245R	N248D	N252K				
V68A	A103V	V1041	G159D	T213E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	T213G	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A215V	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	A215R	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S216T	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S216V	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	S216C	A232V	Q236H	Q245R	N248D	N252K			
G20A	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	N173D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A			A232V					***************************************			
V68A	S103A			Q206R								
												L

V68A	S103A	V104	I G159I	A232V	/ Q236H	1 Q245F	N248I	N252I	:	T		T
V68A	S103A	V104		1	Į.			N252I		1	<b>-</b>	
P55S	V68A	S103A	4		1			N248I				+
V68A	S103A	V104		1				N252K		<del></del>	<del> </del>	<del>                                     </del>
V68A	S103A	V104		1	1			N252K	· ·	<del></del>	1	
V68A	S103A	V1041	G159E	1	1				<del></del>	1		<del> </del>
V68A	S103A	1	G159E	4	·							<del>                                     </del>
V68A	S103A	1	G159D		l l		7					†
V68A	S103A	4	G159D	1	1							<del> </del>
V68A	S103A		G159D			1					<del> </del>	<b> </b>
I8V	V68A	S103A		1	T .	1		N248D				
V68A	S103A	V104I		1	I .	I .		N248D			1	
V68A	S103A	V1041			1	I .		N252K		<del></del>	<del> </del>	$\vdash$
V68A	S103A	V1041		•	4	1		N252K				
V68A	N76D	S103A	1	1	1			N248D	<del></del>	+	<del> </del>	
V68A	S103A	V104I		t	Q245R							
S103A	V104I	G159D	A232S	Q236H	Q245R	N248D	N252K					
V68A	S103A	1	G159D								<u> </u>	
N18S	V68A	S103A	1	1				N248D				
V68A	S103A	V1041	G159D	1			1	N252K				
V68A	N76D	SIOIT	S103A	V104I	G159D	T213R	N218S	A232V	Q236H	Q245R	T260A	
V68A	S103A	V1041	G159D	A228V		i .		N248D				
T33S	V68A	N76D	S103A	V1041				Q245R		N252K		
V68A	N76D	E89D	S103A	V104I				A232V				
G61E	V68A	N76D	S103A	V1041		i		Q245R				
S103A	V1041	G159D		P2101	A232V	Q236H	ı					
G61E	V68A	S103A	V104I	S130A	G159D	A232V	Q236H	Q245R	N248D	N252K		
G61E	V68A	S103A	V1041	A133S	Q137R			Q236H			N252K	
G61E	S103A	V104I	A133V	G159D	A232V			N248D				
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248G	N252K				
V68A	S103A	V104I	G159D	N218S	A232V	Q236H	Q245R	N248D	N252K			
G61E	V68A	S103A	V104I	G159D				Q245R		N252K		
S3L	G61E	V68A	N76D	S103A	V104I			Q245R				
G61E	V68A	S103A	V1041	G159D				Q245R				
G97E	S103A	V104I	G159D			Q245R						
A98D	S103A	V104I				Q245R		N252K				
							02			1		

		,										
S99E	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				
S101E	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				
S101G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				
G102A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V1041	S106E	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V104I	Q109E	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K	N261R				
S103A	V104I	Q109R	G159D	A232V	Q236H	Q245R	N248D	N252K				
N62D	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V1041	G159D	N184D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V104I	G159D	S166D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V1041	G159D	L217E	A232V	Q236H	Q245R	N248D	N252K				
G20R	N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	G159D	Q206R	L217E	A232V	Q236H	Q245R	N248D	N252K			
N62D	S103A	V104I	G159D	Q206R	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	S130G	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V104I	P131V	G159D	A232V	Q236H	Q245R	N248D	N252K				
K27N	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K				
T38G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				
T38A	N76D	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	T260A			
V68A	N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A	E271G		
V68A	N76D	S103A	V104I	G159D	Y209W	T213R	A232V	Q236H	Q245R	T260A		
V68A	N76D	S103A	V104I	G159D	P210I	T213R	A232V	Q236H	Q245R	T260A		
V68A	N76D	S103A	V1041	G159D	V205I	T213R	A232V	Q236H	Q245R	T260A		
V68A	N76D	S103A	V104I	G159D	P210I	A232V	Q236H	Q245R	T260A			
V68A	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A				
N76D	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	T260A			-	
V68A	S103A	V1041	G159D	Y209W	A232V	Q236H	Q245R					
V68A	S103A	V104I	G159D	P210I	A232V	Q236H	Q245R					
V68A	S103A	V104I	G159D	A230V	A232V	Q236H	Q245R					
V68A	S103A	V104I	G159D	L126F	A232V	Q236H	Q245R					
V68A	S103A		G159D								,	
V68A	S103A		G159D									
S103A	V104I			Q236H								
V68A	S103A		G159D			Q245R	T260A					
٠									L	L		

	S103	A V10	4I G1:	59D	A232	V Q236	H Q24:	5R					$\neg$			
	V68.	A S103	A VI		G1591				5H O24	5R L25	7V		$\dashv$		+	
	V68	A S103	A V10	041	G159I		1	2V Q236		5R L25		+	$\dashv$		+-	
	V68/	A S103	A V10	)41	G159I	1	1	V Q236	_	5R L25			$\dashv$		+	
	S103.	A V104	41 G15	9D	A2321	/ Q236	H Q245	R L257	v				$\dashv$		+	
	V68/	4 N761	D S10	3A	V1041	G159	D T213	R A232	V Q23	6H Q24	R T260	A N261	w		+	
	V68/	A S103.	A V10	)41	G159E	A232	V Q236	H Q245		V N261		1.1201			+	
	S103/	A V104	II G15	9D	T213R	A232	V Q236	H Q245	R T260	)A			$\top$			
	S103/	A V104	I G15	9D	P210I	A232	V Q236	H Q245	R N248	D N252	K		$\top$		+-	
	S103A	4 V104	I G15	PD Y	Y209W	/ A232	V Q236	H Q245	R L257	v			十		+	
	V68A		S103	3A	V1041	G1591	P210	L T213	R A232	V Q236	H Q245	R T260	1		+	
	Q12R		4 V10	41 (	G159D	Y209 V	V T2131	R A232	V Q236	H Q245			+		+	
	S103A	V104	I Y209	<u>w</u> /	A232V	Q2361	I Q245	R L257					$\top$		+	_
	S103A	V104	I G159	D.	V2051	P2101	T213I	R A232	V Q236	H Q245	R T260	1	十		_	
	S103A	V1041	I G159	,D	V2051	Y209W	V A2321	V Q2361		R T260			+		<del>                                     </del>	_
	V68A	+	V104	<u> 11   C</u>	3159D	V2051	1	1			H Q245I	2	$\dagger$		<del> </del>	
	S103A	V1041	G159	D '	V2051	Y209W	/ P2101	A2321			R L2571		+		<del> </del>	
į	S103A		G159	DV	V2051	Y209W	/ A232\	/ Q2361		R L257		1	+			
	V68A	S103A	V104	H C	3159D	V2051	Y209V	V P210I	A232	V Q2361	1 Q245R	T260A	+			
	S103A	V1041	G159	<u>D</u>	√205I	Y209W	P210I	A2321		1 Q245F			†			
	S103A	<b>—</b>	G159	DY	209W	P2101	A232V	Q2361	Q245F	2			+			_
	S103A	V1041	G159	<u>D</u> V	/2051	P210I	A232V	Q236H	Q245F	2			$\uparrow$			
1	V68A	S103A	+	<u> 1   S</u>	128L	G159D	A232V	Q236H	Q245F	2		1				_
l	A48V	S103A				A230V	1 1 1 1 1 1						$\top$			٦
-	A48V	V68A	S103A	\\V	1041	G159D	Y209W	A232V	Q236H	Q245R			T			7
r	A48V	V68A	S103A				A232V	f			N252K					$\dashv$
Ī	A48V	V68A	S103A	\\v	1041	G159D	A232V	Q236H	Q245R	L257V	N261W					$\dashv$
_	G102A	S103A	V1041		159D	S212G	A232V	Q236H	Q245R	N248D	N252K		Г			7
		G102A	<del> </del>	_		G159D	S212G	A232V	Q236H	Q245R	N248D	N252K				7
		G102A	S103A	<u>.   v</u>	1041	G159D	ř				N248D					7
	A98L	G102A	S103A	<u> </u>	1041	G159D			1		N248D			_		7
	3102A	S103A	V1041	GI	159D ·	T213R		ł	i	N248D				_		7
	S103A	V1041	P131V	GI	59D /					N252K				$\neg \uparrow$		1
S	103A	V1041	G159D	NI	1					N252K						$\forall$
	103A	V104I	G159D	NI	84G /					N252K						1
S	103A	V1041	G159D	A2	32V C	- 1				N252K						1
								<del>1</del>			<u> </u>					L

STORA   VIOAI   G159D   A232V   Q236H   V244A   Q245R   N248D   N252K   S256R   S103A   VIOAI   G159D   T213R   A232V   Q236H   Q245R   N248D   N252K   S256R   S103A   VIOAI   G159D   N185D   A232V   Q236H   Q245R   N248D   N252K   S256R   S101G   S103A   VIOAI   G159D   N185D   A232V   Q236H   Q245R   N248D   N252K   S256R   S101G   S103A   VIOAI   G159D   Q26E   A232V   Q236H   Q245R   N248D   N252K   S256R   S250R   S250R		<del></del>	<del>,</del>	т						_			
Q12R	S103A	V1041	G159D	A232V	Q236H	V244A	Q245R	N248D	N252K				
Sing   Sing   Sing   Violat   Gispd   Ni85D   A232V   Q236H   Q245R   N248D   N252K   Sing   Sing   Sing   Violat   Gispd   C266E   A232V   Q236H   Q245R   N248D   N252K   Sing   Sing   Sing   Violat   Gispd   C266E   A232V   Q236H   Q245R   N248D   N252K   Sing   C266E   A232V   A232V   Q236H   Q245R   N248D   N252K   Sing   C266E   A232V   A236E   Q245R   N248D   N252K   Sing   C366E   A236E   A	N62D	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K	S256R		
STOTIG  STO3A	Q12R	N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
STOIG   STO3A   V1041   G159D   T213Q   A232V   Q236H   Q245R   N248D   N252K	S101G	S103A	V104I	G159D	N185D	A232V	Q236H	Q245R	N248D	N252K			
A98L   G102A   S103A   V1041   G159D   A232V   Q236H   Q245R   N248D   N252K   N248D   N252K	S101G	S103A	V1041	G159D	Q206E	A232V	Q236H	Q245R	N248D	N252K			
STOTIG  G102A   ST03A   V1041   G159D   A232V   Q236H   Q245R   N248D   N252K   N248D   N252	S101G	S103A	V104I	G159D	T213Q	A232V	Q236H	Q245R	N248D	N252K			
A98L   G102A   S103A   V104I   G159D   S212G   A232V   Q236H   Q245R   N248D   N252K	A98L	G102A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K			
A98L         G102A         S103A         V104I         G159D         S212G         A232V         Q236H         A248D         N252K           N62D         S103A         V104I         G169D         S212G         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         G159D         S212G         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S101G         S103A         V104I         G159D         S212G         T213R         A232V         Q245R         N248D         N252K           S103A         V104I         G159D         A232V         Q245R         N248D         N252K	S101G	G102A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
N62D   S103A   V1041   G159D   T213R   A232V   Q236H   Q245R   N248D   N252K	A98L	G102A	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K		
N62D   S103A   V1041   G159D   S212G   T213R   A232V   Q236H   Q245R   N248D   N252K	A98L	G102A	S103A	V1041	G159D	S212G	A232V	Q236H	N248D	N252K			
N62D   S101G   S103A   V1041   G159D   S212G   T213R   A232V   Q236H   Q245R   N248D   N252K	N62D	S103A	V104I	Q109R	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S103A   V1041   G159D   A232V   Q245R   N248D   N252K	N62D	S103A	V104I	G159D	S212G	T213R	A232V	Q236H	Q245R	N248D	N252K		
S103A   V104I   G159D   A230V   Q245R	N62D	S101G	S103A	V1041	G159D	S212G	T213R	A232V	Q236H	Q245R	N248D	N252K	
N62D         S103A         V104I         S130G         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         S130G         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         S128G         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         S128L         G159D         A232V         Q236H         Q245R         N248D         N252K           N62D         S101G         S103A         V104I         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         S128G         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         S128L         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K <td>S103A</td> <td>V104I</td> <td>G159D</td> <td>A232V</td> <td>Q245R</td> <td>N248D</td> <td>N252K</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	S103A	V104I	G159D	A232V	Q245R	N248D	N252K						
S101G         S103A         V1041         S130G         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V1041         S128G         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V1041         S128L         G159D         A232V         Q236H         Q245R         N248D         N252K           N62D         S101G         S103A         V1041         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V1041         S128G         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V1041         S128L         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V1041         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V1041         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G	S103A	V1041	G159D	A230V	Q245R								
S101G         S103A         V104I         S128G         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         S128L         G159D         A232V         Q236H         Q245R         N248D         N252K           N62D         S101G         S103A         V104I         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         S128G         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         S128L         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S99G         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G	N62D	S103A	V104I	S130G	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S101G         S103A         V1041         S128L         G159D         A232V         Q236H         Q245R         N248D         N252K           N62D         S101G         S103A         V1041         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V1041         S128G         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V1041         S128L         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V1041         S128L         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V1041         G159D         A232V         Q236H         Q245R         N248D         N252K           S99G         S101G         S103A         V1041         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         Y209W         A232V         Q236H         Q245R         N248D         N252K <td>S101G</td> <td>S103A</td> <td>V1041</td> <td>S130G</td> <td>G159D</td> <td>A232V</td> <td>Q236H</td> <td>Q245R</td> <td>N248D</td> <td>N252K</td> <td></td> <td></td> <td></td>	S101G	S103A	V1041	S130G	G159D	A232V	Q236H	Q245R	N248D	N252K			
N62D         S101G         S103A         V104I         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         S128G         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         S128L         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S99G         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         Y209W         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A	S101G	S103A	V104I	S128G	G159D	A232V	Q236H	Q245R	N248D	N252K			
N62D         S103A         V104I         S128G         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           N62D         S103A         V104I         S128L         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K            A98V         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K            S99G         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         S212G         A232V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         Y209W         A232V         Q236H         Q245R         N248D	S101G	S103A	V1041	S128L	G159D	A232V	Q236H	Q245R	N248D	N252K		<u> </u>	
N62D         S103A         V104I         S128L         G159D         T213R         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K         T260A           S101G         S103A         V104I         P131V         G159D         A232V         Q236H         Q245R         N248D         N252K	N62D	S101G	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K         T260A           S101G         S103A         V104I         P131V         G159D         A232V         Q236H         Q245R         N248D         N252K           A98V         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S99G         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         S212G         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         S212G         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I	N62D	S103A	V104I	S128G	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S101G         S103A         V104I         P131V         G159D         A232V         Q236H         Q245R         N248D         N252K           A98V         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S99G         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         S212G         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         Y209W         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I	N62D	S103A	V1041	S128L	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
A98V         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S99G         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         S212G         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         Y209W         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A194P         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I			V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	T260A			
S99G         S101G         S103A         V104I         G159D         A232V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         S212G         A232V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         Y209W         A232V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         A230V         Q236H         Q245R         N248D         N252K            S101G         S103A         V104I         G159D         A194P         A232V         Q236H         Q245R         N248D         N252K            N76D         S101G         S103A         V104I         G159D         A230V         A232V         <													
S101G       S103A       V104I       G159D       S212G       A232V       Q236H       Q245R       N248D       N252K         S101G       S103A       V104I       G159D       Y209W       A232V       Q236H       Q245R       N248D       N252K         S101G       S103A       V104I       G159D       P210I       A232V       Q236H       Q245R       N248D       N252K         S101G       S103A       V104I       G159D       V205I       A232V       Q236H       Q245R       N248D       N252K         S101G       S103A       V104I       G159D       A230V       Q236H       Q245R       N248D       N252K         S101G       S103A       V104I       G159D       A194P       A232V       Q236H       Q245R       N248D       N252K         N76D       S101G       S103A       V104I       G159D       A194P       A232V       Q236H       Q245R       N248D       N252K         S101G       S103A       V104I       G159D       A230V       A232V       Q236H       Q245R       N248D       N252K         S101G       S103A       V104I       G159D       A230V       A232V       Q236H       Q245R       N248D	1 1			V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G       S103A       V104I       G159D       Y209W       A232V       Q236H       Q245R       N248D       N252K				V1041	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         V205I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A194P         A232V         Q236H         Q245R         N248D         N252K           N76D         S101G         S103A         V104I         G159D         A194P         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         A232V         Q236H         Q245R         N248D         N252K	S101G	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K			
S101G         S103A         V104I         G159D         P210I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         V205I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A194P         A232V         Q236H         Q245R         N248D         N252K           N76D         S101G         S103A         V104I         G159D         A194P         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         A232V         Q236H         Q245R         N248D         N252K													
S101G         S103A         V104I         G159D         V205I         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A194P         A232V         Q236H         Q245R         N248D         N252K           N76D         S101G         S103A         V104I         G159D         A194P         A232V         Q236H         Q245R         N248D         N252K           S101G         S103A         V104I         G159D         A230V         A232V         Q236H         Q245R         N248D         N252K			I	T I		A232V	Q236H	Q245R	N248D	N252K			
S101G         S103A         V104I         G159D         A230V         Q236H         Q245R         R         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C				G159D	P210I	A232V	Q236H	Q245R	N248D	N252K		-	
S101G       S103A       V104I       G159D       A194P       A232V       Q236H       Q245R       N248D       N252K         N76D       S101G       S103A       V104I       G159D       A194P       A232V       Q236H       Q245R       N248D       N252K         S101G       S103A       V104I       G159D       A230V       A232V       Q236H       Q245R       N248D       N252K						A232V	Q236H	Q245R	N248D	N252K			
N76D S101G S103A V104I G159D A194P A232V Q236H Q245R N248D N252K S101G S103A V104I G159D A230V A232V Q236H Q245R N248D N252K						Q236H	Q245R						
S101G S103A V104I G159D A230V A232V Q236H Q245R N248D N252K			V104I	G159D	A194P	A232V	Q236H	Q245R	N248D	N252K			
Q2.5A. 142.105 112.2A			S103A	V104I	G159D	A194P	A232V	Q236H	Q245R	N248D	N252K		
N62D   S103A   V104I   G159D   N185D   Q206E   T213R   A232V   Q236H   Q245R   N248D   N252K   E271Q		S103A	V104I	G159D	A230V	A232V	Q236H	Q245R	N248D	N252K			
	N62D	S103A	V104I	G159D	N185D	Q206E	T213R	A232V	Q236H	Q245R	N248D	N252K	E271Q

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table I except for the following substitution sets of Table III:

Table III

						A GUIC II				
76	103	104	259			T	<u> </u>	T	T	T
76	86	103	104					<del> </del>	<del></del>	<del> </del>
76	103	104	130					<del> </del> -	<del> </del>	<del> </del>
76	99	103	104	204				<del></del>	<del> </del>	<del> </del>
76	103	104	242			1	<del>                                     </del>	<del> </del>	<del> </del>	<del> </del>
76	103	104	104	182	198			<del> </del>		+
21	76	103	104	182		<u> </u>		<u> </u>	<del> </del> -	<del>                                     </del>
76	103	104	119	137						-
76	103	104	173	222					<del>                                     </del>	<del> </del>
61	76	103	104	222				<b></b>	<del> </del>	<del> </del>
68	76	103	104	116	159	170	185	232	236	245

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table IV:

Table IV

					1 6	idie I A					
76	103	104	222	245						T	
76	103	104	222	249	<b>-</b>		<del> </del>		<del> </del>	<del> </del>	
68	103	104	159	232	236	245	252				
68	76	103	1.04	159	213	232	236	245	260	<del> </del>	
22	68	76	103	104	<del> </del>		-		200	ļ	
68	103	104	159	232	236	245	248	252		ļ	
68	103	104	159	232	236	245					-
68	103	104	140	159	232	236	245	252			
43	68	103	104	159	232	236	245	252			-
43	68	103	104	159	232	236	245			 	-
12	76	103	104	130	222	245	261				<del> </del>
76	103	104	130	222	245						↓

68	103	104	159	232	236	245	257	T	<u> </u>	T	T
68	76	103	104	159	210	232	236	245		†	<del> </del>
68	103	104	159	224	232	236	245	257		-	<del> </del> -
76	103	104	159	232	236	245	257			<del> </del>	<del> </del>
68	76	103	104	159	211	232	236	245		-	<del> </del>
12	68	76	103	104	159	214	232	236	245	<del>                                     </del>	-
68	76	103	104	159	215	232	236	245	<del> </del>		
12	68	76	103	104	159	232	236	245		<del> </del>	
20	68	76	103	104	159	232	236	245	259		
68	76	87	103	104	159	232	236	245	260		
68	76	103	104	159	232	236	245	261			
12	48	68	76	103	104	159	232	236	245		
76	103	104	159	192	232	236	245				
76	103	104	147	159	232	236	245	248	251		
12	68	76	103	104	159	232	236	245	272		
68	76	103	104	159	183	206	232	236	245		
68	76	103	104	159	232	236	245	256			
68	76	103	104	159	206	232	236	245			
27	68	76	103	104	159	232	236	245			
68	103	104	159	212	232	236	245	248	252		
103	104	159	232	236	245	248	252				
68	103	104	159	209	232	236	245	248	252		
68	103	104	109	159	232	236	245	248	252		
20	68	103	104	159	232	236	245	248	252		
68	103	104	159	209	232	236	245	248	252		
68	103	104	159	210	232	236	245	248	252		
68	103	104	159	212	232	236	245	248	252		
68	103	104	159	213	232	236	245	248	252		
68	103	104	213	232	236	245	248	252			
68	103	104	159	215	232	236	245	248	252		
68	103	104	159	216	232	236	245	248	252		
20	68	103	104	159	232	236	245	248	252		

	103	104	159	232	236	245	0.40				
			1.57	232	230	245	248	252	255		
68	103	104	159	232	236	245	248	252		<u> </u>	<u> </u>
<del></del>		ļi			250	243	240	252	256		
68	103	104	159	232	236	245	248	252	260		<del> </del>
68	103	104	1.50					232	200		1
00	103	104	159	228	232	236	245	248	252	<del>                                     </del>	<del>                                     </del>
68	76	89	103	104	100						1
		67	103	104	159	210	213	232	236	245	260
68	103	104	159	218	222	- 22 -				5	200
			139	210	232	236	245	248	252		

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table V:

Table V

V68	4 5102	A 3/104	LCICOT	T							
7/60	1 3103	A V 104	1 61391	) A228V	A232\	/ Q2361	I Q2451	R N2481	N252K	1	Γ
		A V104		N218S	A232\	/ Q2361	Q2451	R N2481	N252K	1	<del> </del>
L	V68/	1	A V104I	G159D	A2321	/ Q236I	Q245F	N248I	N252K	-	<u> </u>
	N76I	ı	S103A	V104I					/ Q236H		T260A
V68/	S103	A V104	G159D	A232V					S256R		12007
V68A	S103	V1041	G159D	A232V	Q236H	Q245R	N248E	N252k	T260R	ļ	
V68A	S103	V104I	G159D	A232V					T255V		
V68A	S103/	V104I	G159D	A232V			1		S256N		
V68A	S103/	V104I	_L_	A232V	L	Q245R	1	1			
V68A	S103A	V104I	1	T213R	1 -						
V68A	S103A	1	G159D	A215V							
L.	1	V1041		A215R			ì		N252K		
	1	V1041		1 :					N252K		
L	S103A	<u> </u>	<u> </u>	S216T	A232V			1	N252K		
	1			S216V	A232V	Q236H	Q245R	N248D	N252K		
	I	V104I	T213S	A232V	Q236H	Q245R	N248D	N252K			
L	i	V104I	G159D	P210L	A232V	Q236H	Q245R	N248D	N252K	<del></del>	
L	S103A	ŀ	G159D	S212C	A232V	Q236H					
V68A	S103A	V104I	G159D		A232V	Q236H					
S103A	V1041	G159D	A232V	Q236H	Q245R	N248D			-12.52K		
V68A	S103A	V104I	(	Y209W				NOAOD	Nosovi		
		V104I		G159D		Q236H	1	i i	ľ		
		S103A		f		Q236H	t t	,			
				G159D		Q236H	· ·	1			
. 00/1	3103A	V 1041	G159D	Y209F	A232V	Q236H	Q245R	N248D	N252K		

Q12R	N76D	S103A	I104T	S130T	M222S	10245R	N261D		<del></del>	1	<del>-,</del>
	S103A				Q245R	1	112011	<del></del>		ļ	<del>-</del>
	S103A			<u> </u>		ļ	ļ				
N76E		. İ		H249R							
			_i	Q245R	1						
N76D		<u> </u>		L		1	Q245R	1			
N76D			V147I	G159D	A232V	Q236H	Q245R	N248S	K251R		1
Q12R	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	A272S		
V68A	N76D	S103A	V104I	G159D	N183K	Q206L	A232V	Q236H	Q245R		
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	S256R			<del>                                     </del>
V68A	N76D	S103A	V104I	G159D	Q206R	A232V	Q236H	Q245R	<del>  -</del> -		<del>                                     </del>
K27R	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R			-
Q12R	A48V	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R		<del> </del>
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N261W			
V68A	N76D	S103A	V104I	G159D	G211R	A232V	Q236H	Q245R			
V68A	N76D	S103A	V104I	G159D		1	Q236H	1			
Q12R	V68A	N76D	S103A	V104I	L	ł	A232V	İ			
V68A	N76D	S103A	V104I	G159D	ľ		Q236H				
Q12R	V68A	N76D	S103A	V104I	1	1	Q236H				
G20R	V68A	N76D	S103A	V104I		l	Q236H		\$259G		
V68A	N76D	S87R	S103A	V104I		l.	Q236H				
N76D	S103A	V104I	G159D	A232V		L			1200 1	<del></del>	
V68A	N76D	S103A	V104I	G159D			Q236H	Q245R	T260A		
T22K	V68A	N76D	S103A	V104I					120071	<del></del>	
V68A	N76D	S103A	V104I	G159D	P210R	A232V	Q236H	O245R			
	S103A		G159D	S212P			Q245R		Nasay		
L			G159D	ł		1	- 1		NZJZK		
	L !				Q236H		ł	D257 V			
L			G159D	I	Q236H						
i	S103A		G159D				N248D	NOSOV			
			G159D				N248D	N252K			
	S103A		N140D			Q245R	00455	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \			
N43S	V68A			1			Q245R	1			
		S103A					Q245R	N252K			
N43K			1		A232V		1				
N43D	l	S103A					Q245R	N252K			
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	L257V				

A highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12/102/103/104/159/212/232/236/245/248/252; 12/76/103/104/130/170/185/222/243/245; 12/76/103/104/130/222/245/261; 12/76/103/104/130/222/245; 12/76/103/104/222/245; 61/68/103/104/159/232/236/245/248/252; 62/103/104/159/213/232/236/245/248/252; 62/103/104/109/159/213/232/236/245/248/252; 62/103/104/159/232/236/245/248/252; 62/101/103/104/159/212/213/232/236/245/248/252; 62/103/104/130/159/213/232/236/245/248/252; 68/103/104/159/232/236/245/248/252/270; 68/103/104/159/185/232/236/245/248/252; 68/103/104/159/210/232/236/245/248/252; 68/103/104/159/185/210/232/236/245/248/252; 68/103/104/159/213/232/236/245/248/252; 68/103/104/159/230/232/236/245; 68/76/103/104/159/209/232/236/245; 68/103/104/232/236/245/248/257/275; 68/103/104/213/232/236/245/248/252; 68/103/104/159/232/236/245/248/252; 68/103/104/159/209/232/236/245; 68/76/103/104/159/236; 68/76/103/104/159/236/245; 68/76/103/104/159/232/236/245; 68/103/104/159/232/236/245/252; 68/103/104/159/232/236/245; 68/103/104/159/232/236/245/257; 68/76/103/104/159/211/232/236/245; 68/76/103/104/159/215/232/236/245; 68/103/104/159/210/232/236/245; 68/103/104/159/213/232/236/245/260; 68/76/103/104/159/213/232/236/245/260; 68/103/104/159/236; 68/76/103/104/159/210/232/236/245/260; 68/103/104/159/236/245; 68/103/104/159/183/232/236/245/248/252; 68/76/103/104/159/236/245; 68/103/104/232/236/245/257/275; 68/103/104/159/213/232/236/245; 76/103/222/245; 76/103/104/222/245; 76/103/104/159/232/236/245; 76/103/104/159/213/232/236/245/260; 76/103/104/159; 76/103/104/131/159/232/236/245/248/252; 97/103/104/159/232/236/245/248/252; 98/102/103/104/159/212/232/236/245/248/252; 98/103/104/159/232/236/245/248/252; 101/103/104/159/232/236/245/248/252; 102/103/104/159/232/236/245/248/252; 103/104/159/232/236/245; 103/104/159/232/236/245/248/252; 103/104/159/205/209/232/236/245/257 103/104/159/232/245/248/252; 103/104/159/205/209/210/232/236/245/257; 103/104/159/213/232/236/245/248/252; 103/104/159/217/232/236/245/248/252; 103/104/130/159/232/236/245/248/252; 103/104/159/230/236/245; 103/104/159/236/245; 103/104/159/248/252/270; 103/104/131/159/232/236/245/248/252;

103/104/159/232/236/245/257.

103/104/159/205/209/232/236/245; and

PCT/US98/22486

A more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12R/76D/103A/104T/130T/222S/245R: 12R/76D/103A/104I/222S/245R; 12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 12R/76D/103A/104T/130G/222S/245R/261D; 12R/76D/103A/104T/130G/170S/185D/222S/243D/245R; 61E/68A/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K; 62D/103A/104I/159D/213R/232V/236H/245R/248D/252K; 62D/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K; 62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K; 68A/103A/104I/159D/232V/236H/245R/248D/252K/270A; 68A/76D/103A/104I/159D/213R/232V/236H/245R/260A; 68A/103A/104I/159D/236H: 68A/103A/104I/159D/236H/245R; 68A/76D/103A/104I/159D/210I/232V/236H/245R/260A; 68A/103A/104I/159D/183D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/209W/232V/236H/245R; 68A/76D/103A/104I/159D/211R/232V/236H/245R; 68A/76D/103A/104I/159D/215R/232V/236H/245R; 68A/103A/104I/159D/213R/232V/236H/245R/260A; 68A/76D/103A/104I/159D/236H; 68A/76D/103A/104I/159D/236H/245R; 68A/76D/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/252K; 68A/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/257V; 68A/103A/104I/159D/185D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/230V/232V/236H/245R;

68A/76D/103A/104I/159D/209W/232V/236H/245R; 68A/103A/104I/232V/236H/245R/248D/257V/275H; 68A/103A/104I/232V/236H/245R/257V/275H; 68A/103A/104I/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210I/232V/236H/245R; 68A/103A/104I/159D/210L/232V/236H/245R; 68A/103A/104I/159D/213G/232V/236H/245R; 76D/103A/222S/245R; 76D/103A/104I/222S/245R; 76D/103A/104I/159D/232V/236H/245R; 76D/103A/104I/159D; 76D/103A/104I/131V/159D/232V/236H/245R/248D/252K; 76D/103A/104I/159D/213R/232V/236H/245R/260A; 97E/103A/104I/159D/232V/236H/245R/248D/252K; 98L/103A/104I/159D/232V/236H/245R/248D/252K; 98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 101G/103A/104I/159D/232V/236H/245R/248D/252K; 102A/103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/213R/232V/236H/245R/248D/252K; 103A/104I/130G/159D/232V/236H/245R/248D/252K; 103A/104J/159D/230V/236H/245R; 103A/104I/159D/217E/232V/236H/245R/248D/252K; 103A/104I/159D/236H/245R; 103A/104I/159D/248D/252K/270V; 103A/104I/159D/232V/236H/245R; 103A/104I/159D/205I/209W/232V/236H/245R; 103A/104I/159D/232V/236H/245R/257V; 103A/104I/159D/205I/209W/232V/236H/245R/257V; 103A/104I/131V/159D/232V/236H/245R/248D/252K; 103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and103A/104I/159D/232V/245R/248D/252K.

An even more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

```
12/76/103/104/130/222/245/261;
     62/103/104/159/232/236/245/248/252;
   62/103/104/159/213/232/236/245/248/252;
62/101/103/104/159/212/213/232/236/245/248/252;
          68/103/104/159/232/236/245:
       68/103/104/159/230/232/236/245:
       68/103/104/159/209/232/236/245;
       68/103/104/159/232/236/245/257;
    68/76/103/104/159/213/232/236/245/260;
   68/103/104/159/213/232/236/245/248/252;
   68/103/104/159/183/232/236/245/248/252;
   68/103/104/159/185/232/236/245/248/252;
 68/103/104/159/185/210/232/236/245/248/252;
   68/103/104/159/210/232/236/245/248/252;
       68/103/104/159/213/232/236/245:
     98/103/104/159/232/236/245/248/252;
 98/102/103/104/159/212/232/236/245/248/252;
     101/103/104/159/232/236/245/248/252;
     102/103/104/159/232/236/245/248/252;
           103/104/159/230/236/245:
       103/104/159/232/236/245/248/252;
     103/104/159/217/232/236/245/248/252:
    103/104/130/159/232/236/245/248/252;
     103/104/131/159/232/236/245/248/252;
  103/104/159/213/232/236/245/248/252; and
          103/104/159/232/236/245.
```

The most highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

> 12R/76D/103A/104T/130T/222S/245R/261D; 62D/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/159D/213R/232V/236H/245R/248D/252K; 68A/103A/104I/159D/209W/232V/236H/245R; 68A/76D/103A/104I/159D/213R/232V/236H/245R/260A; 68A/103A/104I/159D/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/230V/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/257V; 68A/103A/104I/159D/213G/232V/236H/245R/248D/252K; 68A/103A/104I/159D/185D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/213G/232V/236H/245R: 98L/103A/104I/159D/232V/236H/245R/248D/252K; 98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 101G/103A/104I/159D/232V/236H/245R/248D/252K; 102A/103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/230V/236H/245R; 103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/217E/232V/236H/245R/248D/252K; 103A/104I/130G/159D/232V/236H/245R/248D/252K; 103A/104I/131V/159D/232V/236H/245R/248D/252K; 103A/104I/159D/213R/232V/236H/245R/248D/252K; and 103A/104I/159D/232V/236H/245R.

In another preferred embodiment, the protease variants which are the protease enzymes useful in the cleaning compositions of the present invention comprise protease variants including a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin.

While any combination of the above listed amino acid substitutions may be employed, the preferred protease variant enzymes useful for the present invention comprise the substitution, deletion or insertion of amino acid residues in the following combinations:

- (1) a protease variant including substitutions of the amino acid residues at position 62 and at one or more of the following positions 103, 104, 109, 159, 213, 232, 236, 245, 248 and 252;
- (2) a protease variant including substitutions of the amino acid residues at position 212 and at one or more of the following positions 12, 98, 102, 103, 104, 159, 232, 236, 245, 248 and 252;
- (3) a protease variant including substitutions of the amino acid residues at position 230 and at one or more of the following positions 68, 103, 104, 159, 232, 236 and 245;

- (4) a protease variant including substitutions of the amino acid residues at position 232 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (5) a protease variant including substitutions of the amino acid residues at position 232 and at one or more of the following positions 103, 104, 236 and 245;
- (6) a protease variant including substitutions of the amino acid residues at position 232 and 103 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (7) a protease variant including substitutions of the amino acid residues at position 232 and 104 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (8) a protease variant including substitutions of the amino acid residues at position 232 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (9) a protease variant including substitutions of the amino acid residues at position 232 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (10) a protease variant including substitutions of the amino acid residues at position 232, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (11) a protease variant including substitutions of the amino acid residues at position 252 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (12) a protease variant including substitutions of the amino acid residues at position 252 and at one or more of the following positions 103, 104, 236 and 245;
- (13) a protease variant including substitutions of the amino acid residues at positions 252 and 103 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (14) a protease variant including substitutions of the amino acid residues at positions 252 and 104 and at one or more of the following positions 12, 61, 62, 68, 97, 98,

- 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (15) a protease variant including substitutions of the amino acid residues at positions 252 and 236 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (16) a protease variant including substitutions of the amino acid residues at positions 252 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (17) a protease variant including substitutions of the amino acid residues at positions 252, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270; and
- (18) a protease variant including substitutions of the amino acid residues at position 257 and at one or more of the following positions 68, 103, 104, 205, 209, 210, 232, 236, 245 and 275.

A more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table VI) selected from the group consisting of:

Table VI

76	103	104	212	271							
76	103	104	252	261							
76	103	104	212	258							
4	76	103	104	159	217	252					
12	62	76	103	104	159						
76	103	104	212	268	271						
76	87	103	104	212	271						
76	103	104	212	245	271						
76	103	104	134	141	212	271					
76	103	104	212	236	243	271					
20	62	76	103	104							
68	76	103	104	159	232	236	245				
76	103	104	232	245							
24	68	76	103	104	159	232	236	245			

	T		T		T		<del>,</del>			 	
68	103	104	159	232	236	245	252	<u> </u>			
68	76	103	104	159	213	232	236	245	260		
68	103	104	159	232	236	245	248	252			
68	103	104	159	232	236	245					
68	103	104	140	159	232	236	245	252			
43	68	103	104	159	232	236	245	252			
43	68	103	104	159	232	236	245				
43	68	103	104	159	232	236	245	252			
68	87	103	104	159	232	236	245	252	275		
68	103	104	159	232	236	245	257				
68	103	104	116	159	232	236	245				
68	103	104	159	232	236	245	248				
10	68	103	104	159	232	236	245				
68	103	104	159	203	232	236	245				
68	103	104	159	232	236	237	245				
68	76	79	103	104	159	232	236	245			
68	103	104	159	183	232	236	245				
68	103	104	159	174	206	232	236	245			
68	103	104	159	188	232	236	245				
68	103	104	159	230	232	236	245				
68	98	103	104	159	232	236	245				
68	103	104	159	215	232	236	245				
68	103	104	159	232	236	245	248				
68	76	103	104	159	232	236	245				
68	76	103	104	159	210	232	236	245			
68	76	103	104	159	232	236	245	257			
76	103	104	232	236	245	257					
68	103	104	159	232	236	245	257	275			
76	103	104	257	275					-		
68	103	104	159	224	232	236	245	257			
76	103	104	159	232	236	245	257				
68	76	103	104	159	209	232	236	245			
68	76	103	104	159	211	232	236	245			
12	68	76	103	104	159	214	232	236	245		
68	76	103	104	159	215	232	236	245			
12	68	76	103	104	159	232	236	245			
			<u>-</u> -L							 لـــــا	

20	68	76	103	104	159	232	236	245	5 259	,	$\top$		
68	87	76	103	104	159	232	236	245	5 260		_	$\dashv$	
68	76	103	104	159	232	236	245	_				7	
76	103	3 104	232	236	242	245					$\vdash$	$\dashv$	
68	76	103	104	159	210	232	236	245	;	┪—	$\dashv$	-	
12	48	68	76	103	104	159	232	236	245	<del> </del>	$\neg$	$\dashv$	
76	103	104	232	236	245					<del>                                     </del>			
76	103	.104	159	192	232	236	245			1	+	_	
76	103	104	147	159	232	236	245	248	251	<b>-</b>	1	_	
12	68	76	103	104	159	232	236	245	272	<del> </del>	+	+	·
68	76	103	104	159	183	206	232	236		1	+	$\dashv$	
68	76	103	104	159	232	236	245	256		+-	+-	-	
68	76	103	104	159	206	232	236	245		<del>                                     </del>	+-	+	
27	68	76	103	104	159	232	236	245	1	†	<del>-  </del>	$\dashv$	
68	76	103	104	116	159	170	185	232	236	245	-	十	
61	68	103	104	159	232	236	245	248	252		1	$\top$	
43	68	103	104	159	232	236	245	248	252		1		
68	103	104	159	212	232	236	245	248	252		†		
68	103	104	99	159	184	232	236	245	248	252	†	_	
103	104	159	232	236	245	248	252					_	
68	103	104	159	209	232	236	245	248	252		1	$\top$	
68	103	104	109	159	232	236	245	248	252			1	
20	68	103	104	159	232	236	245	248	252			$\top$	
68	103	104	159	209	232	236	245	248	252			+	
68	103	104	159	232	236	245	248	252	261			+	
68	103	104	159	185	232	236	245	248	252			$\top$	
68	103	104	159	210	232	236	245	248	252				
68	103	104	159	185	210	232	236	245	248	252	1	1	
68	103	104	159	212	232	236	245	248	252			+	
68	103	104	159	213	232	236	245	248	252			$\top$	
68	103	104	213	232	236	245	248	252				1	
68	103	104	159	215	232	236	245	248	252			1	
68	103	104	159	216	232	236	245	248	252			+-	
20	68	103	104	159	232	236	245	248	252			+	$\dashv$
68	103	104	159	173	232	236	245	248	252			+	$\dashv$
68	103	104	159	232	236	245	248	251	252			+-	

68	103	104	159	206	232	236	245	248	252			
68	103	104	159	232	236	245	248	252				
55	68	103	104	159	232	236	245	248	252			
68	103	104	159	232	236	245	248	252	255			
68	103	104	159	232	236	245	248	252	256			
68	103	104	159	232	236	245	248	252	260			
68	103	104	159	232	236	245	248	252	257			1
68	103	104	159	232	236	245	248	252	258			
8	68	103	104	159	232	236	245	248	252	269		
68	103	104	116	159	232	236	245	248	252	260		1
68	103	104	159	232	236	245	248	252	261			
68	103	104	159	232	236	245	248	252	261			
68	76	103	104	159	232	236	245	248	252			
68	103	104	232	236	245	248	252					
103	104	159	232	236	245	248	252					
68	103	104	159	232	236	245	248	252				
18	68	103	104	159	232	236	245	248	252			
68	103	104	159	232	236	245	248	252				
68	76	101	103	104	159	213	218	232	236	245	260	
68	103	104	159	228	232	236	245	248	252			
33	68	76	103	104	159	232	236	245	248	252		
68	76	89	103	104	159	210	213	232	236	245	260	
61	68	76	103	104	159	232	236	245	248	252		
103	104	159	205	210	232	236	245					
61	68	103	104	130	159	232	236	245	248	252		
61	68	103	104	133	137	159	232	236	245	248	252	
61	103	104	133	159	232	236	245	248	252			
68	103	104	159	232	236	245	248	252				
68	103	104	159	218	232	236	245	248	252			
61	68	103	104	159	160	232	236	245	248	252		
3	61	68	76	103	104	232	236	245	248	252	-	
61	68	103	104	159	167	232	236	245	248	252	<del></del>	
97	103	104	159	232	236	245	248	252				
98	103	104	159	232	236	245	248	252				
99	103	104	159	232	236	245	248	252				
101	103	104	159	232	236	245	248	252				

			<del></del>									
102	2 10:	3 10	4 159	232	236	245	248	252	T	T		<del></del>
103	3 104	4 10	6 159	232	236	245	248				<del> </del>	
103	3 104	4 10	9 159	232	236	245	248	252		1		+
103	104	1 159	9 232	236	245	248	252	261			+	+
62	103	3 104	4 159	232	236	245	248	252		1		
103	104	1 159	9 184	232	236	245	248	252	1	<b>-</b>	_	<del> </del>
103	104	159	166	232	236	245	248	252	1	+	+	<del></del>
103	104	159	217	232	236	245	248	252			<del></del>	
20	62	103	104	159	213	232	236	245	248	252	<del>                                     </del>	<del> </del>
62	103	104	159	213	232	236	245	248	252		<del>                                     </del>	<del></del>
103	104	159	206	217	232	236	245	248	252	1	1	<del> </del>
62	103	104	159	206	232	236	245	248	252		<del> </del>	+
103	104	130	159	232	236	245	248	252	T	<del>                                     </del>	<del> </del>	+
103	104	131	159	232	236	245	248	252		<del>                                     </del>	<del> </del>	<del> </del>
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38	76	103	104	159	213	232	236	245	260	<b> </b>		<del> </del>
68	76	103	104	159	213	232	236	245	260	271	<del> </del>	<del> </del>
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68	76	103	104	159	210	232	236	245	260			<del>                                     </del>
68	103	104	159	213	232	236	245	260				
76	103	104	159	213	232	236	245	260				
68	103	104	159	209	232	236	245					
68	103	104	159	210	232	236	245					
68	103	104	159	230	232	236	245					
68	103	104	159	126	232	236	245					
68	103	104	159	205	232	236	245					
68	103	104	159	210	232	236	245					
103	104	159	230	236	245							
68	103	104	159	232	236	245	260					
103	104	159	232	236	245				$\neg \uparrow$			
68	103	104	159	174	232	236	245	257				
68	103	104	159	194	232	236	245	257				—
68	103	104	159	209	232	236	245	257				
										<u>_</u>		

103	104	159	232	236	245	257						
68	76	103	104	159	213	232	236	245	260	261		
68	103	104	159	232	236	245	257	261				
103	104	159	213	232	236	245	260					
103	104	159	210	232	236	245	248	252				
103	104	159	209	232	236	245	257					
68	76	103	104	159	210	213	232	236	245	260		
12	103	104	159	209	213	232	236	245	260			
103	104	209	232	236	245	257						
103	104	159	205	210	213	232	236	245	260		1	
103	104	159	205	209	232	236	245	260				<b>†</b>
68	103	104	159	205	209	210	232	236	245			
103	104	159	205	209	210	232	236	245	257			
103	104	159	205	209	232	236	245	257				
68	103	104	159	205	209	210	232	236	245	260		
103	104	159	205	209	210	232	236	245				
103	104	159	209	210	232	236	245					
103	104	159	205	210	232	236	245					
68	103	104	128	159	232	236	245					
48	103	104	159	230	236	245						
48	68	103	104	159	209	232	236	245				
48	68	103	104	159	232	236	245	248	252			
48	68	103	104	159	232	236	245	257	261			
102	103	104	159	212	232	236	245	248	252			
12	102	103	104	159	212	232	236	245	248	252		
101	102	103	104	159	212	232	236	245	248	252		
98	102	103	104	159	212	232	236	245	248	252		
102	103	104	159	213	232	236	245	248	252			
103	104	131	159	232	236	245	248	252				
103	104	159	184	232	236	245	248	252				
103	104	159	232	236	244	245	248	252				
62	103	104	159	213	232	236	245	248	252	256		
12	62	103	104	159	213	232	236	245	248	252		
101	103	104	159	185	232	236	245	248	252			
101	103	104	159	206	232	236	245	248	252			
101	103	104	159	213	232	236	245	248	252		<del></del>	

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9	3 10	2 10	03 10	4 159	23	2 23	6 24	5 24	8 25	2		
10	1 10	2 10	3 10	4 159	23:		<del></del> -	<del>- </del>			<del>- -</del>	
98	10	2 10	3 10	4 159	21:		<del>-  </del>	-+			_	
98	10	2 10	3 10	4 159	212	2 232					<del>-</del>	
62	10	3 10	4 109	159	213	3 232		+		<del></del>	,  -	<del>-  </del>
62	10:	3 10	4 159	212	213	232		$\neg$	<del></del>			
62	10	10:	3 104	159	212	213	232	<del></del>	<del></del>			
103	104	159	232	245	248	252				1 240	, 2	,,,
103	104	159	230	245						_	+-	
62	103	104	130	159	213	232	236	245	248	252		
101	103	104	130	159	232	236	245	248		<del></del>	+	
101	103	104	128	159	232	236	245	248			-	
62	101	103	104	159	213	232	236	245	248	252	<del> </del>	
62	103	104	128	159	213	232	236	245	248	252	_	
62	103	104	128	159	213	232	236	245	248	252	<del>                                     </del>	
101	103	104	159	232	236	245	248	252	260		1	
101	103	104	131	159	232	236	245	248	252			+
98	101	103	104	159	232	236	245	248	252			<b></b>
99	101	103	104	159	232	236	245	248	252			
101	103	104	159	212	232	236	245	248	252			1
101	103	104	159	209	232	236	245	248	252			<b></b>
101	103	104	159	210	232	236	245	248	252			
101	103	104	159	205	232	236	245	248	252			
101	103	104	159	230	236	245						
101	103	104	159	194	232	236	245	248	252			
76	101	103	104	159	194	232	236	245	248	252		
101 62	103	104	159	230	232	236	245	248	252			
02	103	104	159	185	206	213	232	236	245	248	252	271

An even more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table VII) selected from the group consisting of:

Table VII

- 1						1	able VII					
	N76D	S103A	V1041	S212P	E271V					<u> </u>		ì
	N76D	S103A	V104I	N252K	N261Y							
							<u> </u>	 				ı

_																		
N.	76D S1	03 A	V104	4I S212	2P G25	3R						T	T		T-			_
V	4E N	76D	S103	A V10	4J G159	D L2	17E	N252	D			<del> </del>	+		┼			_
QI	2H N	62H	N761	D S103		41 G1		<del></del>		_		+	<del> </del>	_	+-	$\dashv$		
N7	6D S1	03A	V104	I S212	P V268	F E2	71V			_		+			┼	$\dashv$		_
N7	6D S	37R	S103	A V104	1 S212	P E2	71 V					<del> </del>	+		┼	$\dashv$		_
N7	6D S1	03A	V104	I S212	P Q245	L E2	71V					<del>                                     </del>	+		<del> </del>	$\dashv$		_
N7	6D S1	03A	V104	I T134	S   S141	N S2	12P	E271	7			<del> </del>	+		<del> </del>	$\dashv$		_
N7	6D S1	03A	V104	I S212	P Q236	L N2	43S	E271	,	_		<del>                                     </del>	+			-		_
G2	OV NO	52S	N76D	S103	A V104	1							+-		<del>                                     </del>			_
V6	8A N7	6D	S103A	V104	I G159	D A23	32V	Q2361	1 Q245	R		<del>                                     </del>	┪—					_
N7	5D S10	3A	V1041	A232		- 1							+-			-+		
S24	T V6	8A	N76D	S103A	V104	I G15	9D	A232V	/ Q236	H O24	5R	<del> </del>	<del> </del>			-		_
V68	BA S10	3A	V104I	G159I	A232				N252				+			+		-
V68	3A N7	6D	S103A	V104	G159I					+	 5R	T260A		-		$\dashv$		_
V68	A S10	3A	V1041	1	A232\								+-	-		+		-
V68	A S10	3A	V1041		A2321											$\dashv$		_
V68	A S10	3A	V1041	N140E	G159E	A23	2V	Q236H	Q245I	R N25	2K		<u> </u>	7		+		-
N43	S V68	BA	S103A		G159E	1								$\top$		$\dashv$		-
N43	K V68	A	S103A	1	1	i .	T						1	$\top$		+	<del></del>	_
N43	D V68	A :	S103A	V1041	G159D	A232	2	Q236H	Q245F	N252	2K			$\top$		+		_
V68	A   S87	G !	S103A	V1041	G159D	A232	2V (	Q236H	Q245R	N252	K	R275S		$\top$		_		-
V68	A S103	A	V104I	G159D	A232V	Q236	Н	Q245R	L257V					1		十		1
V68.		A	V104I	NI16D	G159D	A232	v	Q236H	Q245R					$\top$		$\top$		1
V68	_	A \	V104I		A232V									$\top$				1
R100		-+	103A	V1041	G159D	A232	V	Q236Н	Q245R					$\top$		+-		1
V68/	<del></del>		V1041	G159D	V203E	A232	$\mathbf{v} \mathbf{c}$	Q236Н	Q245R					$\top$		+		1
V68/		$\neg$	V104I	G159D	A232V	Q236	Η	C237E	Q245R					$\top$		+		1
V68/			179N	S103A	V104I	G159	DA	1232V	Q236H	Q245	R					1		1
V68/					N183D		v c	)236Н	Q245R					$\top$		1		
V68/		_	/1041	G159D	A174V	Q206	L A	232V	Q236H	Q2451	2					1		
V68A	1		/1041	G159D	S188C	A232	v c	236Н	Q245R					T				İ
V68A	<del> </del>	A V	1041	G159D	A230T	A232	v Q	236H	Q245R					1		1		ĺ
V68A			103A	V104I	G159D	A232\	v Q	236H	Q245R					1		1		
V68A		-	1041	G159D	A215T	A232\	/ Q	236H	Q245R					T		$\vdash$		
V68A	1		1041	G159D	A232V	Q236I	1 Q	245R	N248S			7		$\top$			$\dashv$	
V68A	N76D	SI	03A	V1041	G159D	A232\	/ Q	236Н (	Q245R					$\vdash$		†		
										***				Ц		Ь		

Vé	58A N	76D S1	03A V	1041 G	1590	P210	R A2	32V	022	CII C	245	,				<del></del>	
V6	8A N7	6D S1	03A V	104I G	159D	A232	V 02	361	Q230	SH Q	2431	-			—		<del></del>
N7	6D S16	03A V	041 A2	32V Q	236H	0245	R 126	717	Q24:	OK L.	25 / \	+-			┼		
V6	8A S10	)3A VI		59D A						,, D.		+			<del> </del>		
N7	6D S10	3A V1	04I L2	57V R	275H	1	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	JA	L25 /	VK	2/3H	-			<del> </del>		
V6	8A S10	3A V1		59D T		<del></del>	V 023	6U	0245	D I a		┼			├		
N70	6D S10	3A V1	041 G1:	59D A2	.32V	O2361	H 024	5D	1.257	K L2	3/V	-				$\bot$	
V68	BA N7	5D S10	3A V1	041 G1	59D	Y2091	V A23	2V	0236	V 02	46D			_		$\dashv$	
V68	BA N7	5D S10		041 G1	59D	G211F	R A23	2	Q236	1 02	45R						
V68	BA N76	D S10	3A V10	041 G1	59D	G211V	/ A232	V	0236	1 02	45K						
Q12	R V68	A N7	5D S10	3A V1	041	G159E	Y214	11	Δ2301 Δ2321	V 03	45R						
V68	A N76	D S10.	3A V10	04I G1.	59D	A215R	A232	V	72361	1 02	HOO	Q245	R			——	
Q12	R V68	A N76	D S10	3A V1	041	G159D	A232	V	7236L	1 024	SK		+			4_	
G20	R V68	A N76	D S10				A232					5040	_	$\dashv$		+	
V68	A S87	R N76	D S103	A VI	041	G159D	A232	V	)236H	1 024	5R	S259	G	$\dashv$			
V68/	A N76	D S103	A V10	4I G15	9D /	4232V	Q236	HIC	)245R	N26	3K	1260	<u> </u>	$\dashv$			
V68/	4 N761	S 103	A V10	41 G15	9D A	4232V	0236	НС	)245R	N261	10			$\dashv$			
N76I	S103.	A V104	1 A232	V Q23	- 1	S242P		- 1	LIJK	114201	W			+		┵	
V68.4	N761	S 103	A V104	II G15	9D I	210L	A232\		236H	024	D		<del> </del>	+		+	
Q12F	A481	7 V68	i				G159I		232V	0236	H	12455	,	+		+	
N76E	S103/	4 V104	I A232	V Q23	зн С	245R				Q=30		/243F	+	+		<del> </del>	
N76D	<del>                                     </del>	V104	I G159	D Y19	2F A	232V	Q2361	1 0:	245R		+			╁		<del> </del>	
N76D		V104					Q236H				SK	251R	-	+		├	
Q12R	+	N76E	S103A	V10	‡1 G	159D	A232V	Q2	236H	O245	RA	2725	-	+		-	$\dashv$
V68A	N76D	S103A	V104	G159	DN	183K	Q206L	A2	32V	O236	НО	245R		+-		<del> </del>	$\dashv$
V68A	N76D	S103A	V104	G159	D A	232V	Q236H	Q2	45R	S256F	₹	24510		+			$\dashv$
V68A	N76D	S103A	V1041	G159	D Q	206R	A232V	Q2	36H	Q245I	₹		<del> </del>	+			$\dashv$
K27R	V68A	N76D	S103A				A232V							+-			$\dashv$
V68A	N76D	S103A	<del>                                     </del>	N116	r Gi	59D 1	R170S	N1	85S .	A232\	02	236H	Q245R	-			$\dashv$
G61E	V68A	S103A	V104I	G1591	) A2	32V C	)236Н	Q24	45R 1	N248E	N2	52K	4273K	-			$\dashv$
N43D	V68A	S103A	V104I	G1591	) A2	32V C	)236Н	Q24	15R 1	N248D	N2	52K		-	-	<del></del>	$\dashv$
V68A	S103A	V104I	G159D	S212F	A2	32V C	236Н	Q24	15R N	N248D	N2	52K			$\dashv$		$\dashv$
V68A	S103A	V1041	S99N	G159I	NI	84D A	232V	Q23	6Н С	)245R	N2	48D	N252K				$\dashv$
S103A	V104I	G159D	A232V	Q2361	Q2	45R N	248D	N25	2K				. 12JZK		+		$\dashv$
	S103A	V1041	G159D	Y209W	/ A23	32V Q	236H	Q24	5R N	1248D	N2	52K			+		4
V68A	S103A	V1041	Q109R	G159D	A23	32V Q	236H	Q24	5R N	248D	N2	2K			+		$\dashv$
												-11					┙

	<del></del>	·		· ,									
G20	R V68	A S103	A V104	I G159I	A232	V Q2361	H Q245	R N248	D N252K			$\neg$	
V68	A S103	A V104	I G1591	D Y2091	F A232	V Q2361	1 Q245	R N248	D N252K		1		
V68	A S103	A V104	I G1591	D A232\	/ Q236I	H Q245F	R N248	D N252	K N261D			_	
V68	A S103	A V104	II G1591	N1851	A2321	√ Q2361	1 Q245	R N248	D N252K		†	+	
V68	A S103	A V104		) P210R			- 1	1	D N252K		<del>                                     </del>		
V68	A S103	A V104	I G159I	P210T	A2321	1	i —		N252K	<del></del>			
V68	A S103	A V104	I G159I	P210S	A232\	1			N252K				
V68	A S103/	4 V104	I G159E	N185D	P210L				R N248D		<del>                                     </del>	_	
V68	A S103	4 V104	I G159I	P210L	A232V	/ Q236H			N252K				
V68	A S103	V104	I G159E	S212A		1	į		N252K			+	
V68.	A S103	V104	I G159D	S212G	A232V				N252K			<del> </del>	
V68.	A S103	V104							N252K			+	
V68	A S103A	V104			•		1		N252K			<del></del>	
V68,	A S103A	V1041	T213S	A232V	l .	1		N252K					
V68/	A A103\	/ V1041	G159D	T213E			<b>†</b>		N252K			<del></del> -	
V68/	S103A	V1041	G159D	T213R					N252K		<del></del>	<del>                                     </del>	
V68/	S103A	V104I		1		1		N248D				<del>                                     </del>	$\dashv$
V68/	S103A	V104I	G159D	4					N252K		<del></del>	<del> </del>	$\dashv$
V68/	S103A	V1041							N252K			<del> </del>	ᅱ
V68A	S103A	V1041		1 1		1	·	N248D				<del> </del>	$\dashv$
V68A	S103A	V1041	G159D					N248D	<del></del>			<del> </del> -	$\dashv$
V68A	S103A	V1041	G159D					N248D	<del>                                     </del>		<del></del>	<del> </del>	$\dashv$
G20A	V68A	S103A	V104I			ı .		N248D			<del></del>		ㅓ
V68A	S103A	V1041	G159D			· r		N248D					$\dashv$
V68A	S103A	V1041						K251V					$\dashv$
V68A	S103A	V1041	G159D						N252K				$\dashv$
V68A	S103A	V1041	G159D	i	ı	Q245R		N252F					$\dashv$
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252L					$\dashv$
P55S	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252F			<del></del>	$\exists$
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	T255V				$\dashv$
V68A	S103A	V104I	G159D	A232V		Q245R		N252K	S256N			<del></del>	$\dashv$
V68A	S103A	V104I	G159D	A232V	T	Q245R			S256E				$\dashv$
V68A	S103A	V104I	G159D	A232V	T	Q245R 1			S256R				$\dashv$
V68A	S103A	V1041	G159D	1		Q245R 1			T260R				$\dashv$
V68A	S103A	V104I	G159D					N252K		<del></del>		<del>-</del>	-
V68A	S103A	V104I	G159D	1	1	Q245R 1		N252K				<del></del>	-
						<u> </u>	00		32300				J

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	18V	V68	A S10	3A V	1041	G159	D A	.232	V Q236	H Q	245F	R N248	D N252	K N269	nT	$\neg \neg$	
-	V68/	A S103	A VI	041 N	1168	G159	DA	232	V Q236	H Q	 245F	R N248	D N252	K T260		+-	
-	V68/	A S103	A VI	04I G1	59D	A232			•				K N261		-		
-	V68/	A S103	A V10	04I G1	59D	A232	v Q	236I	H Q245	R N2	248E	N2521	K N261	D			
-	V68/	N76	D S10		1041				V Q236	- 1			D N252			-	
-	V68/	A S103	A VIC	41 A2	32V	Q236	H Q	245F	N248	1					<del></del>	+-	
	S103/	A V104	I G15	9D A2	328	Q236	H Q	245R	N248	D N2	52K					+-	
-	V68A	S103	A V10			1	- 1					N252H		1	+	-	
L	N185	V68/	A S103		041	1			1				N252I		<del> </del>	+	
L	V68A	S103	A V10	41 G1	59D	A2321	v Q	236H	Q245	1		N252K			<del></del>		
Ľ	V68A	N76I	S 101	T S10	)3A	V104	I G	59D	T2131					I Q245R	T260A	+-	
Ļ	V68A	S103	4 V10	41 G1:	59D	A228\		232V				1	N252k		12007	+	
L	T33S	V68A	N76	D S10	)3A	V1041	G1	59D	A232V	1 -			N248E	<del></del>		+-	
	V68A	N76E	E89	) S10	3A	V1041	GI	59D	P210L				Q236H		<del>                                     </del>	+	
1	G61E	V68A	N76	D S10	3A	V1041	Gı	59D	A2321	1 -				N252K		+	
s	103A	V104	G159	D V2	051	P210I	A2	32V	Q2361					INZUZK	<del> </del>	+	
4	361E	V68A	S103.	A VI	041	S130A	G1	59D	A232V	Q23	6H	Q245R	N248D	N252K		-	
4	G61E	V68A	S103.	A V10	041	A133S								N248D		-	
1	361E	S103A	V104	I A13	3V	G159D	A2:	32V	Q236H	Q24	5R	N248D	N252K	1.2.00	112321	<del>                                     </del>	
1	/68A	S103A	V104	1 G15		A232V						N252K		<del> </del>			
	/68A	S103A	V104	I G15	9D	N218S	1				_		N252K			-	
2	661E	V68A	S103/	1 V10	41	G159D	1			1			N248D	N252K		<u> </u>	
1:	S3L	G61E	V68A	N76	D	S103A	Vi		A232V	1	- 1		N248D			<del> </del>	
G	61E	V68A	S103/	V10	41 (	G159D	S16	7F	A232V	1	i		N248D				
G	97E	S103A	V104	G159	D /	A232V	Q23	6Н	Q245R	N248	3D 1	N252K		7.2521			$\dashv$
A	98D	S103A	V104			A232V	ı		Q245R			N252K					$\dashv$
S	99E	S103A	V1041	G159	D A	4232V			Q245R								$\dashv$
SI	01E	S103A	V1041	G159	DA	A232V		- 1	Q245R								$\dashv$
SI	01G	S103A	V104I	G159	DA	1232V		- 1	Q245R								$\dashv$
G1	02A	S103A	V1041	G159	D A	1232V		1	Q245R								$\dashv$
SI	03A	V104I	S106E	G159	DA	- 1		1	Q245R								$\dashv$
SI	03A	V104I	Q109E	G159	DA	1		- 1	Q245R								$\dashv$
S10	03A	V104I	G159D	A232	v Q			1	N248D								$\dashv$
SI	03A	V104I	Q109R	G159		ı		- 1	)245R		_						$\dashv$
N6	2D	S103A	V1041						)245R		_						$\dashv$
S10	03A	V104I	G159D	N1841	DA	- 1			245R								$\dashv$
											7114	LJLK					

S103/	V104	I G159E	S166E	A232V	/ Q2361	I Q2451	R N248	D N252K			T		
S103A	V104	I G159E			1 "			D N252K	<del></del>	<del> </del>	1		
G20R	N62D	S103A	1	1	1			H Q245R	+	N252I	<u> </u>		-
N62D	S103A	V104I	G159E	T213R	1	1		R N248D			+		<del> </del>
S103A	V1041	G159D	1	L217E	1			R N248D			$\top$		
N62D	S103A	V104I	G159D	Q206R	A232V			R N248D	<del></del>				
S103A	V1041	1	I .					N252K			1		
S103A	V1041	1	1	1		T		N252K			十		
K27N	S103A	1	1	i i	1	1		N252K	<del></del>		1		
T38G	S103A	V1041	G159D	A232V	Q236H	Q245R	N248I	N252K			1		<del></del>
T38A	N76D	S103A	V1041	G159D	T213R	A232V	/ Q2361	1 Q245R	T260A				
V68A	N76D	S103A	V1041		1			1 Q245R		<del></del>			
V68A	N76D	S103A	V1041	G159D	i			/ Q236H		<del></del>	+		
V68A	N76D	S103A	V104I	1	1	i		/ Q236H			1		
V68A	N76D	S103A	V1041	G159D	V2051	T213R	A232V	Q236H	Q245R	T260A			
V68A	N76D	S103A	V1041	G159D	P2101	A232V	Q236H	Q245R	T260A				
V68A	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A					
N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A					
V68A	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R						
V68A	S103A	V104I	G159D	P2101	A232V	Q236H	Q245R						
V68A	S103A	V104I	G159D	A230V	A232V	Q236H	Q245R						
V68A	S103A	V104I	G159D	L126F	A232V	Q236H	Q245R						
V68A	S103A	V1041	G159D	V2051	A232V	Q236H	Q245R						
V68A	S103A	V1041		P210L		Q236H	Q245R						
S103A		G159D											
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	T260A						
S103A	V104I	G159D	A232V	Q236H	Q245R								
V68A	S103A	V104I	G159D	A174V	A232V	Q236H	Q245R	L257V					
V68A	S103A	V104I	G159D	A194S	A232V	Q236H	Q245R	L257V					
V68A	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	L257V					
S103A	V104I	G159D	A232V	Q236H	Q245R	L257V							
V68A	N76D	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	T260A	N261W			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V	N261W					
S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	T260A						
S103A	V1041	G159D	P210I	A232V	Q236H	Q245R	N248D	N252K					
S103A	V1041	G159D	Y209W	A232V	Q236H	Q245R	L257V					$\top$	

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124	604 7	17.CD	1	7									_					
		N76D	S103		- 1		D P2	10L 7	2131	R A2	32V	Q2.	36H	Q245	R T26	in A		
		103A	V10	1		Y2091		3R A	2321	/ Q2	36H	Q24	45R	T260	Δ 120	,UA	_	 
		/1041	ı	W A2.	32V	Q2361	H Q24	ISR L	257\	,								 
		1041	G159	D V2	051	P210	T21	3R A	232\	/ Q2	36H	024	15R	T260,				 
		1041	G159	D V2	051	/209V	V A23	2V Q	236F	1 02	45R	T26		1200/	-	-		 
V6	8A S	103A	V104				Y20	- 1	2101					Q2451	-			 
S10	3A V	1041	G159	D V20	)51 Y	'209V	V P21			<del>                                     </del>	$\neg \neg$		_	<u>Q2431</u> L257\		-		 
S10	3A V	1041	G1591				/ A232		36H	024	15R	1.25	71/	L25 /\	<del>-</del>	$\dashv$		 
V68	BA SI	03A	V104	I G15	9D \	/2051	Y209	W P2	101	A 23	27	022	/ /		+	$\dashv$		 
S10	3A V	041	G159I	) V20	51 Y	209W	P210	DI A2	32V	023	2 V	Q230	он (	Q245R	T260	A		 
S103	3A V	041	G159I	Y209	W F	2101	A232	V 02	3611	024	60	Q24:	SK		<del> </del>	$\dashv$		 
\$103	BA VI		G159E				A232	1			$\overline{}$		$\dashv$		<del> </del>	4		 
V68	A SI	03 <i>A</i>	V1041	<del> </del>	- 1		A232	<del></del>		Q24:			-		<del> </del>	$\bot$		
A48	V SI	)3A	V1041	+			Q236			Q24:	OK		+			$\perp$		
A48	V V6		5103A	<del> </del>	- 1				$\overline{}$									
A48	V V6		103A	+	I GI	500	Y2091	W A23	2V	Q236	HIC	245	R			$\perp$		
A48\	V V6		103A	<del> </del>	I GI	50D	A2321	V   Q23	6Н	Q245	RIN	12481	DN	252K		$\perp$		
G102	A S10		/1041	G150	. 63	120	A2321	V   Q23	6H	Q245	R L	.257\	V N	261W		$\perp$		
Q12F			103A	V104	1 61	500	A232\	V Q23	6H	Q245	R N	2481	) N	252K				
S1010	G G 10		103A	V104		500	52120	i   A23:	20/0	Q2361	HQ	245F	N.	248D	N252K			
A98L		<del></del>	103A	V104	C1	500	S212G	A23	2V   C	22361	H Q	245R	N2	248D	N252K			
G102/	A S103			G150F	) m	וטפס	S212G	A232	2V C	2361	1 Q:	245R	N2	48D	N252K			
	V10				12	13K /	A232V	Q236	HIC	)245F	R N	248D	N2	52K				
			500	G159E	A23	2010	⊋236H	Q245	RIN	1248E	N2	252K	_					
S103A	V10	11 (3)	590	N1845	A23	20 0	)236H	Q245	RN	2480	N2	252K						
S103A S103A	V104	I G	500	N184G	A23	2010	)236H	Q245	R N	248D	N2	52K						
S103A	+	-	500	A232V	Q23	6H \	/244T	Q245	RN	248D	N2	52K						_
N62D	S103.		390 /	A232V	Q23	6H   V	244A	Q245	R N	248D	N2	52K						
Q12R			041 (	G159D	T21.	$\frac{3R}{A}$	.232V	Q2361	I Q	245R	N2	48D	N25	2K S	256R			
\$101G		1310	J3A	V1041	G159	PD T	213R	A2321	/ Q:	236H	Q24	45R	N24	8D N	252K		1	$\dashv$
			041 (	3159D	N185	DA	232V	Q236I	1 Q2	245R	N24	18D	N25	2K			+	 $\dashv$
S101G		_	041   0	3159D	Q206	E A	232V	Q2361	I Q2	245R	N24	18D	N25	2K			+	 $\dashv$
S101G		VIVIO	041   G	159D	T213	Q A	232V	Q2361	I Q2	45R	N24	8D	N25	2K			$\dashv$	 $\dashv$
	G102/		3A \	/1041	G159	D A	232V	Q236H	Q2	45R	N24	8D	N25	2K	-		+	 4
S101G			$3A \mid V$	/1041	G159	D A2	232V	Q236H	Q2	45R	N24	8D	N251	)K			-+-	 -
1	G102A	S10:	3A V	1041	G159	D S2	12G /	4232V	02	36H	024	5R 1	N249	D N	531		+	 $\dashv$
A98L	G102A	S103	BA V	1041	G1591	D S2	12G A	1232V	02	2611	N24	21	10.50	NZ	32K		+	 4

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N62I	S103	A V104	I Q109	R G1591	T213	R A232	V 0236	H 0245	R N2481	2 2252	., ]	
N62I	S103,	4 V104	I G1591	D S2120	T213	R A232	0236	H 0245	R N2481	N252	K	
N62I	S1010	G S103.	A V104	I G159I	S2120	G T213F	A232	j j	H Q2451			-
S103/	V104	I G159	D A2321	V Q245F	1	D N2521		1	1 (243)	N248.	D N252	!K
S103A	V104	I G1591	D A230\			7		_	<del></del>	<del></del>	<del> </del>	
N62D	S103A	V104	I S130C	G159E	T213F	R A232V	02361	H 0245	N248E	) N2521		
S101C	S103A	V104		G159D	1				N252K		-	
S101G	S103A	V104	I S128G	G159D	A2321	<del></del>		N248F	) N252V			
S101G	S103A	V104					1		N252K	+	-	
N62D	S101G	S103A	V1041	G159D	T213R		<del>  `                                   </del>		N248D		.	
N62D	S103A	V1041	S128G	G159D	T213R		1	1	N248D			
N62D	S103A	V104I	S128L	G159D	T213R	A232V	<del>                                     </del>	1	N248D		<del>                                     </del>	
S101G	S103A	V1041	G159D	A232V				N252K		N232K	+	
S101G	S103A	V1041	P131V	G159D	A232V				N252K		<del> </del>	
A98V	S101G	S103A	V104I	G159D	A232V	1			N252K	<del></del>		+
S99G	S101G	S103A	V104I	G159D	A232V	1		N248D			<del> </del>	<del> </del>
S101G	S103A	V1041	G159D	S212G	A232V	1		N248D			<del></del>	<del> </del>
S101G	S103A	V104I	G159D	Y209W	A232V							
S101G	S103A	V104I	G159D		A232V	1 1		N248D				
5101G	S103A	V1041	G159D	V2051	A232V	Q236H						<del> </del> -
3101G	S103A	V104I	G159D		Q236H							
101G	S103A	V104I	G159D	A194P	A232V	Q236H	Q245R	N248D	N252K			
N76D	S101G	S103A	V104I		A194P		- 7		N248D	N252K		
101G	S103A	V104I	G159D			Q236H		N248D				
N62D	S103A	V104I	G159D	N185D					Q245R	NI240D	125011	E271Q

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table VI except for the following substitution set of Table VIII:

					Ta	able VII	ľ				
68	76	103	104	116	159	170	185	232	236	245	

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table IX:

Table IX

	60	10	<u> </u>				1	able I	X							
	68	10		04 1	59 2	32 2	236	24		52	T			<del></del>		
L	68	76	5 1	03 1	04 1	59 2	213	23	2 2	36	24	5 26	0	<del> </del>		
	68	10.	3 10	04 1:	59 2	32 2	36	24.	5 2	48	25			<u> </u>	$-\downarrow$	
Γ	68	10.	3 10	04 15	59 2	32 2	36	24:			-				$\dashv$	
	68	103	3 10	04 14	0 1:		32	230		45	252	<del>,  </del>				
	43	68	10	)3 10	14 15		32	236		15						
	43	68	10	3 10			32	236			252	·				
	68	103	10				36			15						
-	68	76	10					245								
-	68	103						232	_		245					
-	76	103						236	_	5	257		7		+-	
-	68	76						245	25	7			$\dashv$		_	
<u> </u>	12		103				1	232	23	6	245		7		+-	
		68	76			1 15	9	214	232	2	236	245	$\dashv$		+-	
L	68	76	103		159	21	5	232	236	5	245	<del> </del>	+			
<u>_</u>	12	68	76	103	104	15	9	232	236	5	245	+	$\dashv$		+-	
<u></u>	20	68	76	103	104	159	9	232	236	;	245	259	+			
	68	76	87	103	104	159	7	232	236	-	245	260	+		+	
(	68	76	103	104	159	232	-	236	245		261		+		$\perp$	
1	12	48	68	76	103	104	+	159	232		236	245	+		<del> </del>	
7	76	103	104	159	192	232	+	236	245	$\perp$		243	_			
7	76	103	104	147	159	232	+	236	245		248	261	$\downarrow$			
1.	2	68	76	103	104	159		232	236			251	_			
6	8	76	103	104	159	183		206	<u>L</u> .	4_	245	272	_			
6	8	76	103	104	159	232		236	232		236	245				
68	8	76	103	104	159	206			245	4_	256					$\neg$
27	7	68	76	103	104			232	236		245					$\neg$
68	8	103	104	159		159	$\bot$	232	236	2	45					$\exists$
10:		104	159		212	232		236	245	2	48	252				$\dashv$
68				232	236	245	2	248	252				_			$\dashv$
		103	104	159	209	232	2	236	245	2.	48	252	_			$\dashv$
68	L	103	104	109	159	232	2	36	245	2	48	252		$\dashv$		-
20		68	103	104	159	232	2	36	245	24	48	252				4

68	103	104	159	209	232	236	245	7 240			
68	103	104	+			1 230	273	248	252		- 1
	103	104	159	210	232	236	245	248	252	+	
68	103	104	159	212	232	236	245	248			
68	103	104	159	213	232				252		
68	103	+			232	236	245	248	252		
	103	104	213	232	236	245	248	252	<del> </del>	+	+
68	103	104	159	215	232	236	245	<b></b>			
68	103	104	150			<u></u>	243	248	252		
			159	216	232	236	245	248	252	<del> </del>	+
20	68	103	104	159	232	236	245	248		<del> </del>	↓
68	103	104	159	232	236				252		
68	103	104			230	245	248	252	255		
	103	104	159	232	236	245	248	252	256	<del> </del>	<del> </del> -
68	103	104	159	232	236	245	240				
68	103	104	159				248	252	260		
			139	228	232	236	245	248	252		<del> </del>
68	76	89	103	104	159	210	213	232	226	0.15	
68	103	104	159	218	232				236	245	260
					232	236	245	248	252		

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table X:

Table X

1 V68	1 5103	A 1/104	1 0 4 4 4			l able	Λ				
1007	1 3103	A V 104	l G159I	A228	V A2321	V Q236	H 0245	R N2481	N252K	1	
V68A	S103	A V104		N218	1		1	1	1	1	
G20R	V68A				1	1 < 0.	H   Q2451	R   N248I	N252K		1
		1	V104I	G1591	A2321	/ Q236I	I Q245I	R N2481	N252K	<del> </del>	├
V68A	N76E	E89D	S103A	V1041	GISOF					i	
V68A	S103A	V1041		1		ı	ı	A232V	1	Q245R	T260
		1	10.00	A232V	/ Q236H	Q245F	N248L	N252K	S256R	<del> </del>	
V68A	S103A	V104I	G159D	A232V			ı	N252K			
V68A	S103A	V104I	L	A232V		1	ı				
	1	1		1	1	Q245R	N248D	N252K	T255V		
VOSA	S103A	V104I	G159D	A232V	Q236H	O245R	N248D	N252K	<b>_</b>		
V68A	S103A	V1041	G159D	A232V		1	1	1	1		
V/69 A	61024	1		1	,	Q245R	N248D	N252L			
VUOA	2103A	V1041	G159D	T213R	A232V	Q236H	O245R	N248D	NOSOV		
V68A	S103A	V104I	G159D	A215V	A222V	022677		112700	N232K		
V68A	S103A	3/10/7			A232V						
		V104I	G159D	A215R	A232V	Q236H	Q245R	N248D	N252K	<del></del> -	
V68A	S103A	V104I	G159D	S216T							
768A	S103A	V104I						N248D			
	ľ		G159D	S216V	A232V	Q236H	Q245R	N248D	N252K		
/68A	S103A	V1041	T213S		Q236H						
L					<b>Q_5011</b>	Q243K	N248D]	N252K			

	V	68A	S10	3A	V104	I GI:	797	T P2 I	OI	142	2217	100											
		58A			V104	ł		1		A23	02 V	Q2:	36H	Q2	45R	N24	8D	N25	2K			T	
		- 1		- 1		I G15				A23	52V	Q23	36H	Q24	45R	N24	8D	N25	2K			+	
						D A23		L								N24	8D	N25	2K			十	
				BA V										N25		1			-			+	
		8A				_L_		Y209		A23		Q23	6Н	Q24	5R	N24	BD	N252	2K			+	
	<u></u>		V68.	L_	/1041	┸`		G159	_ !		ı	Q23	6Н	Q24	5R	N248	BD	N252	k.			$\vdash$	
		8A S			103A	1	- 1	G159	- 1	A232	2V	Q23	6Н	Q24	5R	N248	D	N252	K			┼─	
		1			1041		9D	Y209	9F	A232	2V (	Q23	6H	Q24	5R	N248	D	N252	K			<del> </del>	
	L	6D S			1041	G159	PD	Y192	2F	A232	2V (	Q236	SH	Q24:	5R		$\dashv$		+				
	<u> </u>	SD S			1041		_	G159	D	A232	V C	Q23 <i>6</i>	5H	Q24:	5R	N248	s	K251	R		-		
	Q12	_ 1	V68A		76D	1	A	V104	11	G159	DA	1232	v v	Q23 <i>6</i>	5H	Q245	R	A272			$\dashv$		
		A 1		j	03A	V104	41	G159	D i	N183	K	2206	L,	A232		Q236		Q2451			4		
	V68		176E		03A	V104										S256I			+		$\dashv$		
	V68		176E	ı	03A	V104	II	G1591	D	Q206	R A	232	V	Q236	н	Q2451	1		+		$\dashv$		
	K27		768A	N'	76D	S103.	A	V104								Q245I			+		$\dashv$		
	Q12	R A	48V	Vé	58A	N76I	5 3	5103/		V104						Q236F	- 1	12450	+		$\dashv$		
1	V68	- 1	76D	S10	03A	V104	IC	31591	) A	2321						1261 V		(2451	1		4		
L	V68/	_	76D	1	)3A	V104	i	159[		3211F						245R	_1		+		$\downarrow$		
	V68/	AN	76D	S10	3A	V104	īG	1590		2111				2361	- 1	245R			$\perp$		4		
ľ	Q12F	V	68A	N7	6D	S103A	7	/1041		159E	- 1		1 '			236H	1	245D	$\perp$		$\perp$		
[	V68.A	N'	76D	S10	3A	V1041	G	159D		215R	4			236F	$\perp$	245R	1	243K	ot		$\perp$		
1	Q12F	V	58A	N70	5D	S103A	. T	1041		159D	f		1	236H		245R	1		L.		$\perp$		
7	320R	Ve	58A	N76	5D	S103A	V	1041		159D		32V		_	1 `	245R		259G	_		丄		
1	/68A	N7	76D	S87	'R	S103A	V	1041	. 1	159D			1			245R			_		$\perp$		
N	176D	S10	03A	V10	41 (	G159D	A.	232V	1	236H	1					243K	12	260V			$\perp$		
V	'68A	N7	6D	S103	BA	V104I	G	159D	T2	213R	A2	32V	02	36H	10	2450	777	(0)			$\perp$		╛
٧	68A	N7	6D	S103	A	V104I	GI	59D		10R		32V		36H			12	AUO			$\perp$		
V	68A	S10	3A	V10	4I C	3159D	l .		ı							245R 248D	<del></del>				L		
V	68A	S10	3A	V104	41 0	3159D	T2	24A	A2	32V	023	3611	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	15D	INZ	481)	N2	52K					
		S10		V104		159D	A2			36H	•					3/V					Ĺ		
V	68A	S10	3A	V104		159D	!		_`_		i .	- 1											7
		S10:		V104		159D	!			36H		ı											7
V		S103	- 1	V104						36H			N24	48D	N2.	52K							1
		S103		V104					_	36H		- i											1
	138	V68	$\bot$	\$103/						32V													1
		V68	$\perp$	3103/	L					2V		,		,	N2:	52K		+		$\neg$			1
_				-103/	, v	1041	G15	ַ עפּי	A23	2V	Q23	6Н	Q24	5R				+					1
																							j

				•	A232V			 	
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V	 	

A highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12/102/103/104/159/212/232/236/245/248/252; 61/68/103/104/159/232/236/245/248/252; 62/103/104/130/159/213/232/236/245/248/252; 62/103/104/159/213/232/236/245/248/252; 62/103/104/109/159/213/232/236/245/248/252; 62/103/104/159/232/236/245/248/252; 62/101/103/104/159/212/213/232/236/245/248/252; 68/103/104/159/232/236/245/248/252/270; 68/103/104/159/185/232/236/245/248/252; 68/103/104/159/210/232/236/245/248/252; 68/103/104/159/185/210/232/236/245/248/252; 68/103/104/159/213/232/236/245/248/252; 68/103/104/159/230/232/236/245; 68/76/103/104/159/209/232/236/245; 68/103/104/232/236/245/248/257/275; 68/103/104/213/232/236/245/248/252; 68/103/104/159/232/236/245/248/252; 68/103/104/159/209/232/236/245; 68/76/103/104/159/232/236/245: 68/103/104/159/232/236/245/252; 68/103/104/159/232/236/245: 68/103/104/159/232/236/245/257; 68/76/103/104/159/211/232/236/245; 68/76/103/104/159/215/232/236/245; 68/103/104/159/210/232/236/245; 68/103/104/159/213/232/236/245/260; 68/76/103/104/159/213/232/236/245/260; 68/76/103/104/159/210/232/236/245/260; 68/103/104/159/183/232/236/245/248/252; 68/103/104/232/236/245/257/275; 68/103/104/159/213/232/236/245; 76/103/104/159/232/236/245; 76/103/104/159/213/232/236/245/260; 76/103/104/131/159/232/236/245/248/252; 97/103/104/159/232/236/245/248/252; 98/103/104/159/232/236/245/248/252; 98/102/103/104/159/212/232/236/245/248/252; 101/103/104/159/232/236/245/248/252; 102/103/104/159/232/236/245/248/252; 103/104/159/232/236/245; 103/104/159/248/252/270; 103/104/159/232/236/245/248/252; 103/104/159/205/209/232/236/245/257 103/104/159/232/245/248/252; 103/104/159/205/209/210/232/236/245/257; 103/104/159/213/232/236/245/248/252; 103/104/159/217/232/236/245/248/252; 103/104/130/159/232/236/245/248/252; 103/104/131/159/232/236/245/248/252; 103/104/159/205/209/232/236/245; and 103/104/159/232/236/245/257.

A more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

```
12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;
      61E/68A/103A/104I/159D/232V/236H/245R/248D/252K;
   62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K;
     62D/103A/104I/159D/213R/232V/236H/245R/248D/252K;
       62D/103A/104I/159D/232V/236H/245R/248D/252K;
  62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K;
62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K;
      68A/76D/103A/104I/159D/213R/232V/236H/245R/260A;
      68A/76D/103A/104I/159D/210I/232V/236H/245R/260A;
    68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;
         68A/103A/104I/159D/209W/232V/236H/245R;
       68A/76D/103A/104I/159D/211R/232V/236H/245R;
       68A/76D/103A/104I/159D/215R/232V/236H/245R;
       68A/103A/104I/159D/213R/232V/236H/245R/260A;
          68A/76D/103A/104I/159D/232V/236H/245R;
         68A/103A/104I/159D/232V/236H/245R/252K;
            68A/103A/104I/159D/232V/236H/245R;
         68A/103A/104I/159D/232V/236H/245R/257V;
    68A/103A/104I/159D/185D/232V/236H/245R/248D/252K;
    68A/103A/104I/159D/210L/232V/236H/245R/248D/252K;
 68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K;
    68A/103A/104I/159D/213E/232V/236H/245R/248D/252K;
         68A/103A/104I/159D/230V/232V/236H/245R;
      68A/76D/103A/104I/159D/209W/232V/236H/245R;
      68A/103A/104I/232V/236H/245R/248D/257V/275H;
        68A/103A/104I/232V/236H/245R/257V/275H;
      68A/103A/104I/213E/232V/236H/245R/248D/252K;
      68A/103A/104I/159D/232V/236H/245R/248D/252K;
         68A/103A/104I/159D/210I/232V/236H/245R;
        68A/103A/104I/159D/210L/232V/236H/245R;
        68A/103A/104I/159D/213G/232V/236H/245R;
   68A/103A/104I/159D/232V/236H/245R/248D/252K/270A;
           76D/103A/104I/159D/232V/236H/245R;
   76D/103A/104I/131V/159D/232V/236H/245R/248D/252K;
     76D/103A/104I/159D/213R/232V/236H/245R/260A;
     97E/103A/104I/159D/232V/236H/245R/248D/252K;
     98L/103A/104I/159D/232V/236H/245R/248D/252K;
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98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;
101G/103A/104I/159D/232V/236H/245R/248D/252K;
102A/103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/213R/232V/236H/245R/248D/252K;
103A/104I/130G/159D/232V/236H/245R/248D/252K;
103A/104I/159D/217E/232V/236H/245R/248D/252K;
103A/104I/159D/217E/232V/236H/245R/248D/252K;
103A/104I/159D/232V/236H/245R;
103A/104I/159D/232V/236H/245R;
103A/104I/159D/205I/209W/232V/236H/245R;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and
103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and

Recombinant Proteases/Recombinant Subtilisins - A "recombinant protease" or "recombinant subtilisin" refers to a protease or subtilisin in which the DNA sequence encoding the naturally-occurring protease or subtilisin, respectively, is modified to produce a mutant DNA sequence which encodes the substitution, insertion or deletion of one or more amino acids in the protease or subtilisin amino acid sequence. Suitable modification methods are disclosed herein, and in U.S. Patent Nos. RE 34,606, 5,204,015 and 5,185,258.

Non-Human Proteases/Non-Human Subtilisins - "Non-human proteases" or "non-human subtilisins" and the DNA encoding them may be obtained from many procaryotic and eucaryotic organisms. Suitable examples of procaryotic organisms include gram negative organisms such as *E. coli* or *Pseudomonas* and gram positive bacteria such as *Micrococcus* or *Bacillus*. Examples of eucaryotic organisms from which carbonyl hydrolase and their genes may be obtained include yeast such as *Saccharomyces cerevisiae*, fungi such as *Aspergillus* sp. and non-human mammalian sources such as, for example, *bovine* sp. from which the gene encoding the protease chymosin or subtilisin chymosin can be obtained. A series of proteases and/or subtilisins can be obtained from various related species which have amino acid sequences which are not entirely homologous between the members of that series but which nevertheless exhibit the same or similar type of biological activity. Thus, non-human protease or non-human subtilisin as used herein have a functional definition which refers to proteases or subtilisins, respectively, which are associated, directly or indirectly, with procaryotic and eucaryotic sources.

Variant DNA Sequences - Variant DNA sequences encoding such protease or subtilisin variants are derived from a precursor DNA sequence which encodes a naturallyoccurring or recombinant precursor enzyme. The variant DNA sequences are derived by modifying the precursor DNA sequence to encode the substitution of one or more specific amino acid residues encoded by the precursor DNA sequence corresponding to positions 103 in combination with one or more of the following positions 1, 3, 4, 8, 10, 12, 13, 15, 16, 17, 18, 20, 21, 22, 24, 25, 27, 33, 37, 38, 42, 43, 48, 55, 57, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 115, 116, 117, 119, 121, 123, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 161, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin. Although the amino acid residues identified for modification herein are identified according to the numbering applicable to B. amyloliquefaciens (which has become the conventional method for identifying residue positions in all subtilisins), the preferred precursor DNA sequence useful for the present invention is the DNA sequence of Bacillus lentus as shown in Fig. 3.

In a preferred embodiment, these variant DNA sequences encode the substitution, insertion or deletion of the amino acid residue corresponding to position 103 of *Bacillus amyloliquefaciens* subtilisin in combination with one or more additional amino acid residues corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions

corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of *Bacillus amyloliquefaciens* subtilisin. More preferably, these variant DNA sequences encode the protease variants described herein.

In another preferred embodiment, these variant DNA sequences encode the substitution, insertion or deletion of one or more of the amino acid residues corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin. More preferably, these variant DNA sequences encode the protease variants described herein.

Although the amino acid residues identified for modification herein are identified according to the numbering applicable to *B. amyloliquefaciens* (which has become the conventional method for identifying residue positions in all subtilisins), the preferred precursor DNA sequences useful for the present invention is the DNA sequence of *Bacillus lentus* as shown in Fig. 3.

These recombinant DNA sequences encode protease variants having a novel amino acid sequence and, in general, at least one property which is substantially different from the same property of the enzyme encoded by the precursor protease DNA sequence. Such properties include proteolytic activity, substrate specificity, stability, altered pH profile and/or enhanced performance characteristics.

Specific substitutions corresponding to positions 103 in combination with one or more of the following positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 wherein the numbered positions correspond to the naturallyoccurring subtilisin from Bacillus amyloliquefaciens or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins (such as Bacillus lentus subtilisin) are described herein. Further, specific substitutions corresponding to one or more of the following positions 62, 212, 230, 232, 252 and 257 wherein the numbered positions correspond to the naturally-occurring subtilisin from Bacillus amyloliquefaciens or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins (such as Bacillus lentus subtilisin) are

described herein. These amino acid position numbers refer to those assigned to the mature Bacillus amyloliquefaciens subtilisin sequence presented in Fig. 1. The present invention, however, is not limited to the use of mutation of this particular subtilisin but extends to precursor proteases containing amino acid residues at positions which are "equivalent" to the particular identified residues in Bacillus amyloliquefaciens subtilisin. In a preferred embodiment of the present invention, the precursor protease is Bacillus lentus subtilisin and the substitutions, deletions or insertions are made at the equivalent amino acid residue in B. lentus corresponding to those listed above.

A residue (amino acid) of a precursor protease is equivalent to a residue of *Bacillus amyloliquefaciens* subtilisin if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific residue or portion of that residue in *Bacillus amyloliquefaciens* subtilisin (i.e., having the same or similar functional capacity to combine, react or interact chemically).

In order to establish homology to primary structure, the amino acid sequence of a precursor protease is directly compared to the *Bacillus amyloliquefaciens* subtilisin primary sequence and particularly to a set of residues known to be invariant in subtilisins for which sequence is known. For example, Fig. 2 herein shows the conserved residues as between *B. amyloliquefaciens* subtilisin and *B. lentus* subtilisin. After aligning the conserved residues, allowing for necessary insertions and deletions in order to maintain alignment (i.e., avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of *Bacillus amyloliquefaciens* subtilisin are defined. Alignment of conserved residues preferably should conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues. Conservation of the catalytic triad, Asp32/His64/Ser221 should be maintained.

For example, in Fig. 3 the amino acid sequence of subtilisin from Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis (carlsbergensis) and Bacillus lentus are aligned to provide the maximum amount of homology between amino acid sequences. A comparison of these sequences shows that there are a number of conserved residues contained in each sequence. These conserved residues (as between BPN' and B. lentus) are identified in Fig. 2.

These conserved residues, thus, may be used to define the corresponding equivalent amino acid residues of *Bacillus lentus* (PCT Publication No. WO89/06279 published July 13, 1989), the preferred protease precursor enzyme herein, or the subtilisin referred to as PB92 (EP 0 328 299), which is highly homologous to the preferred *Bacillus lentus* subtilisin. The amino acid sequences of certain of these subtilisins are aligned in Figs. 3A and 3B with the sequence of *Bacillus amyloliquefaciens* subtilisin to produce the maximum

homology of conserved residues. As can be seen, there are a number of deletion in the sequence of *Bacillus lentus* as compared to *Bacillus amyloliquefaciens* subtilisin. Thus, for example, the equivalent amino acid for Vall65 in *Bacillus amyloliquefaciens* subtilisin in the other subtilisins is isoleucine for *B. lentus and B. licheniformis*. Thus, for example, the amino acid at position +76 is asparagine (N) in both *B. amyloliquefaciens* and *B. lentus subtilisins*. In the protease variants of the invention, however, the amino acid equivalent to +76 in *Bacillus amyloliquefaciens* subtilisin is substituted with aspartate (D). The abbreviations and one letter codes for all amino acids in the present invention conform to the Patentin User Manual (GenBank, Mountain View, CA) 1990, p. 101.

"Equivalent residues" may also be defined by determining homology at the level of tertiary structure for a precursor protease whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the precursor protease and *Bacillus amyloliquefaciens* subtilisin (N on N, CA on CA, C on C and O on O) are within 0.13nm and preferably 0.1nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the protease in question to the *Bacillus amyloliquefaciens* subtilisin. The best model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available.

$$R factor = \frac{\sum_{h} |Fo(h)| - |Fc(h)|}{\sum_{h} |Fo(h)|}$$

Equivalent residues which are functionally analogues to a specific residue of Bacillus amyloliquefaciens subtilisin are defined as those amino acids of the precursor protease which may adopt a conformation such that they either alter, modify or contribute to protein structure, substrate binding or catalysis in a manner defined and attributed to a specific residue of the Bacillus amyloliquefaciens subtilisin. Further, they are those residues of the precursor protease (for which a tertiary structure has been obtained by x-ray crystallography) which occupy an analogous position to the extent that, although the main chain atoms of the given residue may not satisfy the criteria of equivalence on the basis of occupying a homologous position, the atomic coordinates of at least two fo the side chain atoms of the residue lie with 0.13nm of the corresponding side chain atoms of Bacillus amyloliquefaciens subtilisin. The coordinates of the three dimensional structure of Bacillus amyloliquefaciens subtilisin are set forth in EPO Publication No. 0 251 446 (equivalent to US Patent 5,182,204, the disclosure of which is incorporated herein by reference) and can be used as outlined above to determine equivalent residues on the level of tertiary structure.

Some of the residues identified for substitution, insertion or deletion are conserved residues whereas others are not. In the case of residues which are not conserved, the replacement of one or more amino acids is limited to substitutions which produce a variant which has an amino acid sequence that does not correspond to one found in nature. In the case of conserved residues, such replacements should not result in natural-occurring sequence. The protease variants of the present invention include the mature forms of protease variants, as well as the pro- and pre-pro-forms of such protease variants. The prepro-forms are the preferred construction since this facilitates the expression, secretion and maturation of the protease variants.

"Prosequence" refers to a sequence of amino acids bound to the N-terminal portion of the mature form of a protease which when removed results in the appearance of the "mature" form of the protease. Many proteolytic enzymes are found in nature as translational proenzyme products and, in the absence of post-translational processing, are expressed in this fashion. A preferred prosequence for producing protease variants is the putative prosequence of *Bacillus amyloliquefaciens* subtilisin, although other protease prosequences may be used.

A "signal sequence" or "presequence" refers to any sequence of amino acids bound to the N'terminal portion of a protease or to the N-terminal portion of a proprotease which may participate in the secretion of the mature or pro forms of the protease. This definition of signal sequence is a functional one, meant to include all those amino sequences encoded by the N-terminal portion of the protease gene which participate in the effectuation of the secretion of protease under native conditions. The present invention utilizes such sequences to effect the secretion of the protease variants as defined here. One possible signal sequence comprises the first seven amino acid residues of the signal sequence from Bacillus subtilis subtilisin fused to the remainder of the signal sequence of the subtilisin from Bacillus lentus (ATCC 21536).

A "prepro" form of a protease variant consists of the mature form of the protease having a prosequence operably linked to the amino terminus of the protease and a "pre" or "signal" sequence operably linked to the amino terminus of the prosequence.

"Expression vector" refers to a DNA construct containing a DNA sequence which is operably linked to a suitable control sequence capable of effecting the expression of said DNA in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently or the host genome, or may, in some instances, integrate into the

genome itself. In the present specification, "plasmid" and "vector" are sometimes used interchangeably as the plasmid is the most commonly used form of vector at present. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which are, or become, known in the art.

The "host cells" used in the present invention generally are procaryotic or eucaryotic hosts which preferably have been manipulated by the methods disclosed in US Patent RE 34,606 to render them incapable of secreting enzymatically active endoprotease. A preferred host cell for expressing protease is the *Bacillus* strain BG2036 which is deficient in enzymatically active neutral protease and alkaline protease (subtilisin). The construction of strain BG2036 is described in detail in US Patent 5,264,366. Other host cells for expressing protease include *Bacillus subtilis* 168 (also described in US Patent RE 34,606 and US Patent 5,264,366, the disclosure of which are incorporated herein by reference), as well as any suitable *Bacillus* strain such as *B. licheniformis*, *B. lentus*, etc.).

Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the protease variants or expressing the desired protease variant. In the case of vectors which encode the pre- or prepro-form of the protease variant, such variants, when expressed, are typically secreted from the host cell in to the host cell medium.

"Operably linked, "when describing the relationship between two DNA regions, simply means that they are functionally related to each other. For example, a prosequence is operably linked to a peptide if it functions as a signal sequence, participating in the secretion of the mature form of the protein most probably involving cleavage of the signal sequence. A promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation.

The genes encoding the naturally-occurring precursor protease may be obtained in accord with the general methods known to those skilled in the art. The methods generally comprise synthesizing labeled probes having putative sequences encoding regions of the protease of interest, preparing genomic libraries from organisms expressing the protease, and screening the libraries for the gene of interest by hybridization to the probes. Positively hybridizing clones are then mapped and sequenced.

The cloned protease is then used to transform a host cell in order to express the protease. The protease gene is then ligated into a high copy number plasmid. This plasmid replicates in hosts in the sense that it contains the well-known elements necessary for plasmid replication: a promote operably linked to the gene in question (which may be supplied as the gene's own homologous promoter if it is recognized, i.e. transcribed by the host), a transcription termination and polyadenylation region (necessary for stability of the

mRNA transcribed by the host from the protease gene in certain eucaryotic host cells) which is exogenous or is supplied by the endogenous terminator region of the protease gene and, desirably, a selection gene such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antibioticcontaining media. High copy number plasmids also contain an origin of replication for the host, thereby enabling large numbers of plasmids to be generated in the cytoplasm without chromosomal limitation. However, it is within the scope herein to integrate multiple copies of the protease gene into host genome. This is facilitated by procaryotic and eucaryotic organisms which are particularly susceptible to homologous recombination. The gene can be a natural B. lentus gene. Alternatively, a synthetic gene encoding a naturallyoccurring or mutant precursor protease may be produced. In such an approach, the DNA and/or amino acid sequence of the precursor protease is determined. Multiple, overlapping synthetic single-stranded DNA fragments are thereafter synthesized, which upon hybridization and ligation produce a synthetic DNA enclding the precursor protease. An example of synthetic gene construction is set forth in Example 3 of US Patent 5,204,105, the disclosure of which is incorporated herein by reference.

Once the naturally-occurring or synthetic precursor protease gene has been cloned, a number of modifications are undertaken to enhance the use of the gene beyond synthesis of the naturally-occurring precursor protease. Such modifications include the production of recombinant proteases as disclosed in US Patent RE 34,606 and EPO Publication No. 0 251 446 and the production of protease variants described herein.

The following cassette mutagenesis method may be used to facilitate the construction of the proteases variants of the present invention, although other methods may be used. First, the naturally-occurring gene encoding the protease is obtained and sequenced in whole or in part. Then the sequence is scanned for a point at which it is desired to make a mutation (deletion, insertion or substitution) of one or more amino acids in the encoded enzyme. The sequences flanking this point are evaluated for the presence of restriction sites for replacing a short segment of the gene with an oligonucleotide pool which, when expressed will encode various mutants. Such restriction sites are preferably unique sites within the protease gene so as to facilitate the replacement of the gene segment. However, any convenient restriction site which is not overly redundant in the protease gene may be used, provided the gene fragments generated by restriction digestion can be reassembled in proper sequence. If restriction sites are not present at locations within a convenient distance from the selected point (from 10 to 15 nucleotides), such sites are generated by substituting nucleotides in the gene in such fashion that neither the reading frame nor the amino acids encoded are changed in the final construction. Mutation of the gene in order to change its sequence to conform to the desired sequence is accomplished by

M13 primer extension in accord with generally known methods. The task of locating suitable flanking regions and evaluating the needed changes to arrive at two convenient restriction site sequences is made routine by the redundancy of the genetic code, a restriction enzyme map of the gene and the large number of different restriction enzymes. Note that if a convenient flanking restriction site if available, the above method need be used only in connection with the flanking region which does not contain a site.

Once the naturally-occurring DNA or synthetic DNA is cloned, the restriction sites flanking the positions to be mutated are digested with the cognate restriction enzymes and a plurality of end termini-complementary oligonucleotide cassettes are ligated into the gene. The mutagenesis is simplified by this method because all of the oligonucleotides can be synthesized so as to have the same restriction sites, and no synthetic linkers are necessary to create the restriction sites. As used herein, proteolytic activity is defined as the rate of hydrolysis of peptide bonds per milligram of active enzyme. Many well known procedures exist for measuring proteolytic activity (K. M. Kalisz, "Microbial Proteinases," Advances in Biochemical Engineering/Biotechnology, A. Fiechter ed., 1988). In addition to or as an alternative to modified proteolytic activity, the variant enzymes of the present invention may have other modified properties such as  $K_m$ ,  $k_{cat}$ ,  $k_{cat}$ / $K_m$  ratio and/or modified substrate specifically and/or modified pH activity profile. These enzymes can be tailored for the particular substrate which is anticipated to be present, for example, in the preparation of peptides or for hydrolytic processes such as laundry uses.

In one aspect of the invention, the objective is to secure a variant protease having altered proteolytic activity as compared to the precursor protease, since increasing such activity (numerically larger) enables the use of the enzyme to more efficiently act on a target substrate. Also of interest are variant enzymes having altered thermal stability and/or altered substrate specificity as compared to the precursor. In some instances, lower proteolytic activity may be desirable, for example a decrease in proteolytic activity would be useful where the synthetic activity of the proteases is desired (as for synthesizing peptides). One may wish to decrease this proteolytic activity, which is capable of destroying the product of such synthesis. Conversely, in some instances it may be desirable to increase the proteolytic activity of the variant enzyme versus its precursor. Additionally, increases or decreases (alteration) of the stability of the variant, whether alkaline or thermal stability, may be desirable. Increases or decreases in  $k_{cat}$ ,  $K_m$  or  $K_{cat}$ / $K_m$  are specific to the substrate used to determine these kinetic parameters.

In another aspect of the invention, it has been determined that substitutions at positions corresponding to 103 in combination with one or more of the following positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111,

114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin are important in modulating overall stability and/or proteolytic activity of the enzyme.

In a further aspect of the invention, it has been determined that substitutions at one or more of the following positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin are also important in modulating overall stability and/or proteolytic activity of the enzyme.

These substitutions are preferably made in *Bacillus lentus* (recombinant or native-type) subtilisin, although the substitutions may be made in any *Bacillus* protease.

Based on the screening results obtained with the variant proteases, the noted mutations in *Bacillus amyloliquefaciens* subtilisin are important to the proteolytic activity, performance and/or stability of these enzymes and the cleaning or wash performance of such variant enzymes.

Methods and procedures for making the enzymes used in the detergent and cleaning compositions of the present invention are known and are disclosed in PCT Publication No. WO 95/10615.

The enzymes of the present invention have trypsin-like specificity. That is, the enzymes of the present invention hydrolyze proteins by preferentially cleaving the peptide bonds of charged amino acid residues, more specifically residues such as arginine and lysine, rather than preferentially cleaving the peptide bonds of hydrophobic amino acid residues, more specifically phenylalanine, tryptophan and tyrosine. Enzymes having the latter profile have a chymotrypsin-like specificity. Substrate specificity as discussed above is illustrated by the action of the enzyme on two synthetic substrates. Protease's having trypsin-like specificity hydrolyze the synthetic substrate bVGR-pNA preferentially over the synthetic substrate sucAAPF-pNA. Chymotrypsin-like protease enzymes, in contrast, hydrolyze the latter much faster than the former. For the purposes of the present invention the following procedure was employed to define the trypsin-like specificity of the protease enzymes of the present invention:

A fixed amount of a glycine buffer at a pH of 10 and a temperature of 25 °C is added to a standard 10 ml test tube. 0.5 ppm of the active enzyme to be tested is added to the test tube. Approximately, 1.25 mg of the synthetic substrate per mL of buffer solution is added to the test tube. The mixture is allowed to incubate for 15 minutes at 25 °C. Upon completion of the incubation period, an enzyme inhibitor, PMSF, is added to the mixture at

a level of 0.5 mg per mL of buffer solution. The absorbency or OD value of the mixture is read at a 410 nm wavelength. The absorbence then indicates the activity of the enzyme on the synthetic substrate. The greater the absorbence, the higher the level of activity against that substrate.

To then determine the specificity of an individual enzyme, the absorbence on the two synthetic substrate proteins may be converted into a specificity ratio. For the purposes of the present invention, the ratio is determined by the formula specificity of:

[activity on sAAPF-pNA]/[activity on bVGR-pNA]

An enzyme having a ratio of less than about 10, more preferably less than about 5 and most preferably less than about 2.5 may then be considered to demonstrate trypsin-like activity.

Such variants generally have at least one property which is different from the same property of the protease precursor from which the amino acid sequence of the variant is derived.

One aspect of the invention are compositions, such as detergent and cleaning compositions, for the treatment of textiles, dishware, tableware, kitchenware, cookware, and other hard surface substrates that include one or more of the variant proteases of the present invention. Protease-containing compositions can be used to treat for example: silk or wool, as well as other types of fabrics, as described in publications such as RD 216,034, EP 134,267, US 4,533,359, and EP 344,259; and dishware, tableware, kitchenware, cookware, and other hard surface substrates as described in publications such as in US 5,478,742, US 5,346,822, US 5,679,630, and US 5,677,272.

II. Amylase Variants - The amylase variants used in the present invention include, but are not limited to, the amylase enzymes described in WO 95/26397 and in WO 96/23873 (Novo). These enzymes are incorporated into cleaning compositions at a level of from about 0.0001%, preferably from about 0.00018%, more preferably from about 0.00024%, most preferably from about 0.05% to about 0.1%, preferably to about 0.060%, more preferably to about 0.048% by weight of the cleaning compositions of pure enzyme.

The amylase variants are preferably selected from the group consisting of  $\alpha$ -amylase variants.

Suitable  $\alpha$ -amylase variants for use in the present invention include, but are not limited to the following  $\alpha$ -amylases:

(i)  $\alpha$ -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas®  $\alpha$ -amylase activity assay and/or;

- (ii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv)  $\alpha$ -amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or:
- (v)  $\alpha$ -amylase according to (i-iv) wherein the  $\alpha$ -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi)  $\alpha$ -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii)  $\alpha$ -amylase showing positive immunological cross-reactivity with antibodies raised against an  $\alpha$ -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent  $\alpha$ -amylase, wherein the parent  $\alpha$ -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an  $\alpha$ -amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an  $\,\alpha$ amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent \(\alpha\)-amylase has been deleted; and/or (B) at least one amino acid residue of said parent  $\alpha$ -amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent  $\alpha$ -amylase; said variant having an  $\alpha$ -amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or  $\alpha$ -amylolytic activity at neutral to relatively high pH values; increased  $\alpha$ -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pl) so as to better match the pl value for  $\alpha$ -amylase variant to the pH of the medium.

A polypeptide is considered to be X% homologous to the parent amylase if a comparison of the respective amino acid sequences, performed via algorithms, such as the one described by Lipman and Pearson in Science 227, 1985, p. 1435, reveals an identity of X%.

In the context of the present invention, the term "obtainable from" is intended not only to indicate an amylase produced by a *Bacillus* strain but also an amylase encoded by a DNA sequence isolated from such a *Bacillus* strain and produced in a host organism transformed with the DNA sequence.

III. <u>Protease/Amylase Combination</u> - Although any one or more of the protease variants described above can be combined with one or more of the amylase variants described above, in a highly preferred embodiment of the present invention, the protease variant comprises the substitution set: 101/103/104/159/232/236/245/248/252, and more highly preferred the substitution set: 101G/103A/104I/232V/236H/245R/248D/252K.

Although the protease variant and amylase variant can be present in the cleaning compositions in any ratio by ppm, a preferred ratio of protease variant(s) to amylase variant(s) by ppm in the cleaning compositions of the present invention are in the range of from about 1:20 to about 20:1, preferably from about 1:10 to about 10:1, more preferably from about 1:3 to about 3:1.

## **CLEANING COMPOSITIONS**

The cleaning compositions of the present invention also comprise, in addition to one or more protease variants described hereinbefore, one or more cleaning adjunct materials, preferably compatible with the protease variant(s). The term "cleaning adjunct materials", as used herein, means any liquid, solid or gaseous material selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid; granule; powder; bar; paste; spray; tablet; gel; foam composition), which materials are also preferably compatible with the protease enzyme used in the composition. Granular compositions can also be in "compact" form and the liquid compositions can also be in a "concentrated" form.

The specific selection of cleaning adjunct materials are readily made by considering the surface, item or fabric to be cleaned, and the desired form of the composition for the cleaning conditions during use (e.g., through the wash detergent use). The term "compatible", as used herein, means the cleaning composition materials do not reduce the proteolytic activity of the protease enzyme to such an extent that the protease is not effective as desired during normal use situations. Examples of suitable cleaning adjunct materials include, but are not limited to, surfactants, builders, bleaches, bleach activators, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dispersants, suds suppressors, dyes, perfumes, colorants, filler salts, hydrotropes, photoactivators, fluorescers, fabric conditioners, hydrolyzable surfactants, perservatives, anti-oxidants, anti-shrinkage agents, anti-wrinkle agents, germicides, fungicides, color speckles, silvercare, anti-tarnish and/or anti-corrosion agents, alkalinity sources, solubilizing agents, carriers, processing aids,

pigments and pH control agents as described in U.S. Patent Nos. 5,705,464, 5,710,115, 5,698,504, 5,695,679, 5,686,014 and 5,646,101. Specific cleaning composition materials are exemplified in detail hereinafter.

If the cleaning adjunct materials are not compatible with the protease variant(s) in the cleaning compositions, then suitable methods of keeping the cleaning adjunct materials and the protease variant(s) separate (not in contact with each other) until combination of the two components is appropriate can be used. Suitable methods can be any method known in the art, such as gelcaps, encapulation, tablets, physical separation, etc.

Preferably an effective amount of one or more protease variants described above are included in compositions useful for cleaning a variety of surfaces in need of proteinaceous stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form and including both granular and liquid automatic dishwashing); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations); and denture cleaning compositions, unlimited in form (e.g., liquid, tablet).

As used herein, "effective amount of protease variant" refers to the quantity of protease variant described hereinbefore necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like.

Preferably the cleaning compositions comprise from about 0.0001%, preferably from about 0.001%, more preferably from about 0.01% by weight of the cleaning compositions of one or more protease variants of the present invention, to about 10%, preferably to about 1%, more preferably to about 0.1%. Also preferably the protease variant of the present invention is present in the compositions in an amount sufficient to provide a ratio of mg of active protease per 100 grams of composition to ppm theoretical Available O₂ ("AvO₂") from any peroxyacid in the wash liquor, referred to herein as the Enzyme to Bleach ratio (E/B ratio), ranging from about 1:1 to about 20:1. Several examples of various cleaning compositions wherein the protease variants of the present invention may be employed are discussed in further detail below. Also, the cleaning compositions may include from about 1% to about 99.9% by weight of the composition of the cleaning adjunct materials.

The cleaning compositions of the present invention may be in the form of "fabric cleaning compositions" or "non-fabric cleaning compositions."

As used herein, "fabric cleaning compositions" include hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the soaking and/or pretreatment of stained fabrics.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing detergent compositions, oral cleaning compositions, denture cleaning compositions and personal cleaning compositions.

When the cleaning compositions of the present invention are formulated as compositions suitable for use in a laundry machine washing method, the compositions of the present invention preferably contain both a surfactant and a builder compound and additionally one or more cleaning adjunct materials preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. Laundry compositions can also contain softening agents, as additional cleaning adjunct materials.

The compositions of the present invention can also be used as detergent additive products in solid or liquid form. Such additive products are intended to supplement or boost the performance of conventional detergent compositions and can be added at any stage of the cleaning process.

When formulated as compositions for use in manual dishwashing methods the compositions of the invention preferably contain a surfactant and preferably other cleaning adjunct materials selected from organic polymeric compounds, suds enhancing agents, group II metal ions, solvents, hydrotropes and additional enzymes.

If needed the density of the laundry detergent compositions herein ranges from 400 to 1200 g/litre, preferably 500 to 950 g/litre of composition measured at 20°C.

The "compact" form of the cleaning compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt; inorganic filler salts are conventional ingredients of detergent compositions in powder form; in conventional detergent compositions, the filler salts are present in substantial amounts, typically 17-35% by weight of the total composition. In the compact compositions, the filler salt is present in amounts not exceeding 15% of the total composition, preferably not exceeding 10%, most preferably not exceeding 5% by weight of the composition. The inorganic filler salts, such as meant in the present compositions are selected from the alkali and alkaline-earth-metal salts of sulfates and chlorides. A preferred filler salt is sodium sulfate.

Liquid cleaning compositions according to the present invention can also be in a "concentrated form", in such case, the liquid cleaning compositions according the present invention will contain a lower amount of water, compared to conventional liquid detergents. Typically the water content of the concentrated liquid cleaning composition is preferably less than 40%, more preferably less than 30%, most preferably less than 20% by weight of the cleaning composition.

## Cleaning Adjunct Materials

<u>Surfactant System</u> - Detersive surfactants included in the fully-formulated cleaning compositions afforded by the present invention comprises at least 0.01%, preferably at least about 0.1%, more preferably at least about 0.5%, most preferably at least about 1% to about 60%, more preferably to about 35%, most preferably to about 30% by weight of cleaning composition depending upon the particular surfactants used and the desired effects.

The detersive surfactant can be nonionic, anionic, ampholytic, zwitterionic, cationic, semi-polar nonionic, and mixtures thereof, nonlimiting examples of which are disclosed in U.S. Patent Nos. 5,707,950 and 5,576,282. Preferred detergent and cleaning compositions comprise anionic detersive surfactants or mixtures of anionic surfactants with other surfactants, especially nonionic surfactants.

Nonlimiting examples of surfactants useful herein include the conventional  $C_{11}$ - $C_{18}$  alkyl benzene sulfonates and primary, secondary and random alkyl sulfates, the  $C_{10}$ - $C_{18}$  alkyl alkoxy sulfates, the  $C_{10}$ - $C_{18}$  alkyl polyglycosides and their corresponding sulfated polyglycosides,  $C_{12}$ - $C_{18}$  alpha-sulfonated fatty acid esters,  $C_{12}$ - $C_{18}$  alkyl and alkyl phenol alkoxylates (especially ethoxylates and mixed ethoxy/propoxy),  $C_{12}$ - $C_{18}$  betaines and sulfobetaines ("sultaines"),  $C_{10}$ - $C_{18}$  amine oxides, and the like. Other conventional useful surfactants are listed in standard texts.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated such that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

Nonionic Surfactants - Polyethylene, polypropylene, and polybutylene oxide condensates of alkyl phenols are suitable for use as the nonionic surfactant of the surfactant systems of the present invention, with the polyethylene oxide condensates being preferred. Commercially available nonionic surfactants of this type include Igepal TM CO-630, marketed by the GAF Corporation; and Triton TM X-45, X-114, X-100 and X-102, all marketed by the Rohm & Haas Company. These surfactants are commonly referred to as alkylphenol alkoxylates (e.g., alkyl phenol ethoxylates).

The condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethylene oxide are suitable for use as the nonionic surfactant

of the nonionic surfactant systems of the present invention. Examples of commercially available nonionic surfactants of this type include TergitolTM 15-S-9 (the condensation product of C₁₁-C₁₅ linear alcohol with 9 moles ethylene oxide), TergitolTM 24-L-6 NMW (the condensation product of C₁₂-C₁₄ primary alcohol with 6 moles ethylene oxide with a narrow molecular weight distribution), both marketed by Union Carbide Corporation; NeodolTM 45-9 (the condensation product of C₁₄-C₁₅ linear alcohol with 9 moles of ethylene oxide), NeodolTM 23-3 (the condensation product of C₁₂-C₁₃ linear alcohol with 3.0 moles of ethylene oxide), NeodolTM 45-7 (the condensation product of C₁₄-C₁₅ linear alcohol with 7 moles of ethylene oxide), NeodolTM 45-5 (the condensation product of C₁₄-C₁₅ linear alcohol with 5 moles of ethylene oxide) marketed by Shell Chemical Company, KyroTM EOB (the condensation product of C₁₃-C₁₅ alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company, and Genapol LA O3O or O5O (the condensation product of C₁₂-C₁₄ alcohol with 3 or 5 moles of ethylene oxide) marketed by Hoechst. Preferred range of HLB in these products is from 8-11 and most preferred from 8-10.

Also useful as the nonionic surfactant of the surfactant systems of the present invention are the alkylpolysaccharides disclosed in U.S. Patent No. 4,565,647.

Preferred alkylpolyglycosides have the formula:  $R^2O(C_nH_{2n}O)_t(glycosyl)_x$  wherein  $R^2$  is selected from the group consisting of alkyl, alkylphenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures thereof in which the alkyl groups contain from about 10 to about 18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, preferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 2.7.

The condensation products of ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol are also suitable for use as the additional nonionic surfactant systems of the present invention. Examples of compounds of this type include certain of the commercially-available Plurafac TM LF404 and Pluronic TM surfactants, marketed by BASF.

Also suitable for use as the nonionic surfactant of the nonionic surfactant system of the present invention, are the condensation products of ethylene oxide with the product resulting from the reaction of propylene oxide and ethylenediamine. Examples of this type of nonionic surfactant include certain of the commercially available TetronicTM compounds, marketed by BASF.

Preferred for use as the nonionic surfactant of the surfactant systems of the present invention are polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethylene oxide, alkylpolysaccharides, and mixtures thereof. Most preferred are C₈-C₁₄ alkyl phenol

ethoxylates having from 3 to 15 ethoxy groups and  $C_8$ - $C_{18}$  alcohol ethoxylates (preferably  $C_{10}$  avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula:  $R^2 - C(O) - N(R^1) - Z$  wherein  $R^1$  is H, or  $R^1$  is  $C_{1-4}$  hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof,  $R^2$  is  $C_{5-31}$  hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably,  $R^1$  is methyl,  $R^2$  is a straight  $C_{11-15}$  alkyl or  $C_{16-18}$  alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

Anionic Surfactants - Suitable anionic surfactants to be used are linear alkyl benzene sulfonate, alkyl ester sulfonate surfactants including linear esters of C₈-C₂₀ carboxylic acids (i.e., fatty acids) which are sulfonated with gaseous SO₃ according to "The Journal of the American Oil Chemists Society", 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm oil, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprise alkyl ester sulfonate surfactants of the structural formula:

wherein  $R^3$  is a  $C_8$ - $C_{20}$  hydrocarbyl, preferably an alkyl, or combination thereof,  $R^4$  is a  $C_1$ - $C_6$  hydrocarbyl, preferably an alkyl, or combination thereof, and M is a cation which forms a water soluble salt with the alkyl ester sulfonate. Suitable salt-forming cations include metals such as sodium, potassium, and lithium, and substituted or unsubstituted ammonium cations, such as monoethanolamine, diethanolamine, and triethanolamine. Preferably,  $R^3$  is  $C_{10}$ - $C_{16}$  alkyl, and  $R^4$  is methyl, ethyl or isopropyl. Especially preferred are the methyl ester sulfonates wherein  $R^3$  is  $C_{10}$ - $C_{16}$  alkyl.

Other suitable anionic surfactants include the alkyl sulfate surfactants which are water soluble salts or acids of the formula ROSO₃M wherein R preferably is a  $C_{10}$ - $C_{24}$  hydrocarbyl, preferably an alkyl or hydroxyalkyl having a  $C_{10}$ - $C_{20}$  alkyl component, more preferably a  $C_{12}$ - $C_{18}$  alkyl or hydroxyalkyl, and M is H or a cation. Typically, alkyl chains of  $C_{12}$ - $C_{16}$  are preferred for lower wash temperatures (e.g. below about 50°C) and  $C_{16}$ -18 alkyl chains are preferred for higher wash temperatures (e.g. above about 50°C).

Other anionic surfactants useful for detersive purposes include salts of soap,  $C_{8}$ - $C_{22}$  primary of secondary alkanesulfonates,  $C_{8}$ - $C_{24}$  olefinsulfonates, sulfonated

polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in British patent specification No. 1,082,179,  $C_8$ - $C_{24}$  alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide); alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinates (especially saturated and unsaturated  $C_{12}$ - $C_{18}$  monoesters) and diesters of sulfosuccinates (especially saturated and unsaturated  $C_{6}$ - $C_{12}$  diesters), acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, and alkyl polyethoxy carboxylates such as those of the formula  $RO(CH_2CH_2O)_k$ - $CH_2COO$ -M+ wherein R is a  $C_8$ - $C_{22}$  alkyl, k is an integer from 1 to 10, and M is a soluble salt-forming cation. Resin acids and hydrogenated resin acids are also suitable, such as rosin, hydrogenated rosin, and resin acids and hydrogenated resin acids present in or derived from tall oil.

Further examples are described in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perry and Berch). A variety of such surfactants are also generally disclosed in U.S. Patent 3,929,678, issued December 30, 1975 to Laughlin, et al. at Column 23, line 58 through Column 29, line 23 (herein incorporated by reference).

Highly preferred anionic surfactants include alkyl alkoxylated sulfate surfactants hereof are water soluble salts or acids of the formula RO(A)_mSO3M wherein R is an unsubstituted C₁₀-C₂₄ alkyl or hydroxyalkyl group having a C₁₀-C₂₄ alkyl component, preferably a  $C_{12}$ - $C_{20}$  alkyl or hydroxyalkyl, more preferably  $C_{12}$ - $C_{18}$  alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like. Exemplary surfactants are  $C_{12}$ - $C_{18}$  alkyl polyethoxylate (1.0) sulfate ( $C_{12}$ - $C_{18}E(1.0)M$ ),  $C_{12}$ - $C_{18}$  alkyl polyethoxylate (2.25) sulfate ( $C_{12}$ - $C_{18}$ E(2.25)M),  $C_{12}$ - $C_{18}$  alkyl polyethoxylate (3.0) sulfate ( $C_{12}$ - $C_{18}E(3.0)M$ ), and  $C_{12}-C_{18}$  alkyl polyethoxylate (4.0) sulfate ( $C_{12}-C_{18}E(4.0)M$ ), wherein M is conveniently selected from sodium and potassium.

When included therein, the cleaning compositions of the present invention typically comprise from about 1%, preferably from about 3% to about 40%, preferably about 20% by weight of such anionic surfactants.

Cationic Surfactants - Cationic detersive surfactants suitable for use in the cleaning compositions of the present invention are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyltrimethylammonium halogenides, and those surfactants having the formula:  $[R^2(OR^3)_y][R^4(OR^3)_y]_2R^5N+X-\text{ wherein }R^2\text{ is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each <math>R^3$  is selected from the group consisting of -CH₂CH₂-, -CH₂CH(CH₃)-, -CH₂CH(CH₂OH)-, -CH₂CH₂CH₂-, and mixtures thereof; each  $R^4$  is selected from the group consisting of  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_4$  hydroxyalkyl, benzyl ring structures formed by joining the two  $R^4$  groups, -CH₂CHOH-CHOHCOR⁶CHOHCH₂OH wherein  $R^6$  is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when y is not 0;  $R^5$  is the same as  $R^4$  or is an alkyl chain wherein the total number of carbon atoms of  $R^2$  plus  $R^5$  is not more than about 18; each y is from 0 to about 10 and the sum of the y values is from 0 to about 15; and X is any compatible anion.

Highly preferred cationic surfactants are the water-soluble quaternary ammonium compounds useful in the present composition having the formula (i):  $R_1R_2R_3R_4N^+X^-$  wherein  $R_1$  is  $C_8$ - $C_{16}$  alkyl, each of  $R_2$ ,  $R_3$  and  $R_4$  is independently  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_4$  hydroxy alkyl, benzyl, and - $(C_2H_{40})_xH$  where x has a value from 2 to 5, and X is an anion. Not more than one of  $R_2$ ,  $R_3$  or  $R_4$  should be benzyl. The preferred alkyl chain length for  $R_1$  is  $C_{12}$ - $C_{15}$  particularly where the alkyl group is a mixture of chain lengths derived from coconut or palm kernel fat or is derived synthetically by olefin build up or OXO alcohols synthesis. Preferred groups for  $R_2R_3$  and  $R_4$  are methyl and hydroxyethyl groups and the anion X may be selected from halide, methosulfate, acetate and phosphate ions.

Examples of suitable quaternary ammonium compounds of formulae (i) for use herein are include, but are not limited to: coconut trimethyl ammonium chloride or bromide; coconut methyl dihydroxyethyl ammonium chloride or bromide; decyl triethyl ammonium chloride; decyl dimethyl hydroxyethyl ammonium chloride or bromide; C₁₂₋₁₅ dimethyl hydroxyethyl ammonium chloride or bromide; coconut dimethyl hydroxyethyl ammonium chloride or bromide; myristyl trimethyl ammonium methyl sulphate; lauryl dimethyl benzyl ammonium chloride or bromide; lauryl dimethyl (ethenoxy)₄ ammonium chloride or bromide; choline esters (compounds of formula (i) wherein R₁ is CH₂-CH₂-O-C-C₁₂₋₁₄ alkyl and R₂R₃R₄ are methyl); and di-alkyl imidazolines [(i)].

II

Other cationic surfactants useful herein are also described in U.S. Patent 4,228,044, Cambre, issued October 14, 1980 and in European Patent Application EP 000,224.

When included therein, the cleaning compositions of the present invention typically comprise from about 0.2%, preferably from about 1% to about 25%, preferably to about 8% by weight of such cationic surfactants.

Ampholytic Surfactants - Ampholytic surfactants, examples of which are described in U.S. Patent No. 3,929,678, are also suitable for use in the cleaning compositions of the present invention.

When included therein, the cleaning compositions of the present invention typically comprise from about 0.2%, preferably from about 1% to about 15%, preferably to about 10% by weight of such ampholytic surfactants.

Zwitterionic Surfactants - Zwitterionic surfactants, examples of which are described in U.S. Patent No. 3,929,678, are also suitable for use in cleaning compositions.

When included therein, the cleaning compositions of the present invention typically comprise from about 0.2%, preferably from about 1% to about 15%, preferably to about 10% by weight of such zwitterionic surfactants.

<u>Semi-polar Nonionic Surfactants</u> - Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides having the formula:

↑

R³(OR⁴)_xN(R⁵)₂

wherein R³ is an alkyl, hydroxyalkyl, or alkyl phenyl group or mixtures thereof containing from about 8 to about 22 carbon atoms; R⁴ is an alkylene or hydroxyalkylene group containing from about 2 to about 3 carbon atoms or mixtures thereof; x is from 0 to about 3; and each R⁵ is an alkyl or hydroxyalkyl group containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups (the R⁵ groups can be attached to each other, e.g., through an oxygen or nitrogen atom, to form a ring structure); water-soluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

The amine oxide surfactants in particular include  $C_{10}$ - $C_{18}$  alkyl dimethyl amine oxides and  $C_{8}$ - $C_{12}$  alkoxy ethyl dihydroxy ethyl amine oxides.

When included therein, the cleaning compositions of the present invention typically comprise from about 0.2%, preferably from about 1% to about 15%, preferably to about 10% by weight of such semi-polar nonionic surfactants.

Cosurfactants - The cleaning compositions of the present invention may further comprise a cosurfactant selected from the group of primary or tertiary amines. Suitable primary amines for use herein include amines according to the formula R₁NH₂ wherein R₁ is a C₆-C₁₂, preferably C₆-C₁₀ alkyl chain or R₄X(CH₂)_n, X is -O-,-C(O)NH- or -NH-, R₄ is a C₆-C₁₂ alkyl chain n is between 1 to 5, preferably 3. R₁ alkyl chains may be straight or branched and may be interrupted with up to 12, preferably less than 5 ethylene oxide moieties.

Preferred amines according to the formula herein above are n-alkyl amines. Suitable amines for use herein may be selected from 1-hexylamine, 1-octylamine, 1-decylamine and laurylamine. Other preferred primary amines include C8-C10 oxypropylamine, octyloxypropylamine, 2-ethylhexyl-oxypropylamine, lauryl amido propylamine and amido propylamine. The most preferred amines for use in the compositions herein are 1-hexylamine, 1-octylamine, 1-decylamine, 1-dodecylamine. Especially desirable are n-dodecyldimethylamine and bishydroxyethylcoconutalkylamine and oleylamine 7 times ethoxylated, lauryl amido propylamine and cocoamido propylamine.

LFNIs - Particularly preferred surfactants in the automatic dishwashing compositions (ADD) of the present invention are low foaming nonionic surfactants (LFNI) which are described in U.S. Patent Nos. 5,705,464 and 5,710,115. LFNI may be present in amounts from 0.01% to about 10% by weight, preferably from about 0.1% to about 10%, and most preferably from about 0.25% to about 4%. LFNIs are most typically used in ADDs on account of the improved water-sheeting action (especially from glass) which they confer to the ADD product. They also encompass non-silicone, nonphosphate polymeric materials further illustrated hereinafter which are known to defoam food soils encountered in automatic dishwashing.

Preferred LFNIs include nonionic alkoxylated surfactants, especially ethoxylates derived from primary alcohols, and blends thereof with more sophisticated surfactants, such as the polyoxypropylene/polyoxyethylene/polyoxypropylene (PO/EO/PO) reverse block polymers as described in U.S. Patent Nos. 5,705,464 and 5,710,115.

LFNIs which may also be used include those POLY-TERGENT® SLF-18 nonionic surfactants from Olin Corp., and any biodegradable LFNI having the melting point properties discussed hereinabove.

These and other nonionic surfactants are well known in the art, being described in more detail in Kirk Othmer's Encyclopedia of Chemical Technology, 3rd Ed., Vol. 22, pp. 360-379, "Surfactants and Detersive Systems", incorporated by reference herein.

Bleaching System - The cleaning compositions of the present invention preferably comprise a bleaching system. Bleaching systems typically comprise a "bleaching agent" (source of hydrogen peroxide) and an "initiator" or "catalyst". When present, bleaching agents will typically be at levels of from about 1%, preferably from about 5% to about 30%, preferably to about 20% by weight of the composition. If present, the amount of bleach activator will typically be from about 0.1%, preferably from about 0.5% to about 60%, preferably to about 40% by weight, of the bleaching composition comprising the bleaching agent-plus-bleach activator.

Bleaching Agents - Hydrogen peroxide sources are described in detail in the herein incorporated Kirk Othmer's Encyclopedia of Chemical Technology, 4th Ed (1992, John Wiley & Sons), Vol. 4, pp. 271-300 "Bleaching Agents (Survey)", and include the various forms of sodium perborate and sodium percarbonate, including various coated and modified forms.

The preferred source of hydrogen peroxide used herein can be any convenient source, including hydrogen peroxide itself. For example, perborate, e.g., sodium perborate (any hydrate but preferably the mono- or tetra-hydrate), sodium carbonate peroxyhydrate or equivalent percarbonate salts, sodium pyrophosphate peroxyhydrate, urea peroxyhydrate, or sodium peroxide can be used herein. Also useful are sources of available oxygen such as persulfate bleach (e.g., OXONE, manufactured by DuPont). Sodium perborate monohydrate and sodium percarbonate are particularly preferred. Mixtures of any convenient hydrogen peroxide sources can also be used.

A preferred percarbonate bleach comprises dry particles having an average particle size in the range from about 500 micrometers to about 1,000 micrometers, not more than about 10% by weight of said particles being smaller than about 200 micrometers and not more than about 10% by weight of said particles being larger than about 1,250 micrometers. Optionally, the percarbonate can be coated with a silicate, borate or water-soluble surfactants. Percarbonate is available from various commercial sources such as FMC, Solvay and Tokai Denka.

Compositions of the present invention may also comprise as the bleaching agent a chlorine-type bleaching material. Such agents are well known in the art, and include for example sodium dichloroisocyanurate ("NaDCC"). However, chlorine-type bleaches are less preferred for compositions which comprise enzymes.

(a) Bleach Activators - Preferably, the peroxygen bleach component in the composition is formulated with an activator (peracid precursor). The activator is present at

levels of from about 0.01%, preferably from about 0.5%, more preferably from about 1% to about 15%, preferably to about 10%, more preferably to about 8%, by weight of the composition. Preferred activators are selected from the group consisting of tetraacetyl ethylene diamine (TAED), benzoylcaprolactam (BzCL), 4-nitrobenzoylcaprolactam, 3-chlorobenzoylcaprolactam, benzoyloxybenzenesulphonate (BOBS), nonanoyloxybenzenesulphonate (NOBS), phenyl benzoate (PhBz), decanoyloxybenzenesulphonate (C10-OBS), benzoylvalerolactam (BZVL), octanoyloxybenzenesulphonate (C8-OBS), perhydrolyzable esters and mixtures thereof, most preferably benzoylcaprolactam and benzoylvalerolactam. Particularly preferred bleach activators in the pH range from about 8 to about 9.5 are those selected having an OBS or VL leaving group.

Preferred hydrophobic bleach activators include, but are not limited to, nonanoyloxybenzenesulphonate (NOBS), 4-[N-(nonaoyl) amino hexanoyloxy]-benzene sulfonate sodium salt (NACA-OBS) an example of which is described in U.S. Patent No. 5,523,434, dodecanoyloxybenzenesulphonate (LOBS or C₁₂-OBS), 10-undecenoyloxybenzenesulfonate (UDOBS or C₁₁-OBS with unsaturation in the 10 position), and decanoyloxybenzoic acid (DOBA).

Preferred bleach activators are those described in U.S. 5,698,504 Christie et al., issued December 16, 1997; U.S. 5,695,679 Christie et al. issued December 9, 1997; U.S. 5,686,401 Willey et al., issued November 11, 1997; U.S. 5,686,014 Hartshorn et al., issued November 11, 1997; U.S. 5,405,412 Willey et al., issued April 11, 1995; U.S. 5,405,413 Willey et al., issued April 11, 1995; U.S. 5,130,045 Mitchel et al., issued July 14, 1992; and U.S. 4,412,934 Chung et al., issued November 1, 1983, and copending patent applications U. S. Serial Nos. 08/709,072, 08/064,564, all of which are incorporated herein by reference.

The mole ratio of peroxygen bleaching compound (as AvO) to bleach activator in the present invention generally ranges from at least 1:1, preferably from about 20:1, more preferably from about 10:1 to about 1:1, preferably to about 3:1.

Quaternary substituted bleach activators may also be included. The present cleaning compositions preferably comprise a quaternary substituted bleach activator (QSBA) or a quaternary substituted peracid (QSP); more preferably, the former. Preferred QSBA structures are further described in U.S. 5,686,015 Willey et al., issued November 11, 1997; U.S. 5,654,421 Taylor et al., issued August 5, 1997; U.S. 5,460,747 Gosselink et al., issued October 24, 1995; U.S. 5,584,888 Miracle et al., issued December 17, 1996; and U.S. 5,578,136 Taylor et al., issued November 26, 1996; all of which are incorporated herein by reference.

Highly preferred bleach activators useful herein are amide-substituted as described in U.S. 5,698,504, U.S. 5,695,679, and U.S. 5,686,014 each of which are cited herein above. Preferred examples of such bleach activators include: (6-octanamidocaproyl)

oxybenzenesulfonate, (6-nonanamidocaproyl)oxybenzenesulfonate, (6-decanamidocaproyl)oxybenzenesulfonate and mixtures thereof.

Other useful activators, disclosed in U.S. 5,698,504, U.S. 5,695,679, U.S. 5,686,014 each of which is cited herein above and U.S. 4,966,723Hodge et al., issued October 30, 1990, include benzoxazin-type activators, such as a  $C_6H_4$  ring to which is fused in the 1,2-positions a moiety --C(O)OC(R¹)=N-.

Depending on the activator and precise application, good bleaching results can be obtained from bleaching systems having with in-use pH of from about 6 to about 13, preferably from about 9.0 to about 10.5. Typically, for example, activators with electron-withdrawing moieties are used for near-neutral or sub-neutral pH ranges. Alkalis and buffering agents can be used to secure such pH.

Acyl lactam activators, as described in U.S. 5,698,504, U.S. 5,695,679 and U.S. 5,686,014, each of which is cited herein above, are very useful herein, especially the acyl caprolactams (see for example WO 94-28102 A) and acyl valerolactams (see U.S. 5,503,639 Willey et al., issued April 2, 1996 incorporated herein by reference).

- (b) Organic Peroxides, especially Diacyl Peroxides These are extensively illustrated in Kirk Othmer, Encyclopedia of Chemical Technology, Vol. 17, John Wiley and Sons, 1982 at pages 27-90 and especially at pages 63-72, all incorporated herein by reference. If a diacyl peroxide is used, it will preferably be one which exerts minimal adverse impact on spotting/filming.
- (c) Metal-containing Bleach Catalysts The present invention compositions and methods may utilize metal-containing bleach catalysts that are effective for use in bleaching compositions. Preferred are manganese and cobalt-containing bleach catalysts.

One type of metal-containing bleach catalyst is a catalyst system comprising a transition metal cation of defined bleach catalytic activity, such as copper, iron, titanium, ruthenium tungsten, molybdenum, or manganese cations, an auxiliary metal cation having little or no bleach catalytic activity, such as zinc or aluminum cations, and a sequestrate having defined stability constants for the catalytic and auxiliary metal cations, particularly ethylenediaminetetraacetic acid, ethylenediaminetetra (methylenephosphonic acid) and water-soluble salts thereof. Such catalysts are disclosed in U.S. 4,430,243 Bragg, issued February 2, 1982.

Manganese Metal Complexes - If desired, the compositions herein can be catalyzed by means of a manganese compound. Such compounds and levels of use are well known in the art and include, for example, the manganese-based catalysts disclosed in U.S. Patent Nos. 5,576,282; 5,246,621; 5,244,594; 5,194,416; and 5,114,606; and European Pat. App. Pub. Nos. 549,271 A1, 549,272 A1, 544,440 A2, and 544,490 A1; Preferred examples of these catalysts include MnIV₂(u-O)₃(1,4,7-trimethyl-1,4,7-triazacyclononane)₂(PF₆)₂,

Mn^{III}₂(u-O)₁(u-OAc)₂(1,4,7-trimethyl-1,4,7-triazacyclononane)₂(ClO₄)₂, Mn^{IV}₄(u-O)₆(1,4,7-triazacyclononane)₄(ClO₄)₄, Mn^{III}_{Mn}_{IV}₄(u-O)₁(u-OAc)₂-(1,4,7-trimethyl-1,4,7-triazacyclononane)₂(ClO₄)₃, Mn^{IV}(1,4,7-trimethyl-1,4,7-triazacyclononane)₂(OCH₃)₃(PF₆), and mixtures thereof. Other metal-based bleach catalysts include those disclosed in U.S. Patent Nos. 4,430,243 and U.S. 5,114,611. The use of manganese with various complex ligands to enhance bleaching is also reported in the following: U.S. Patent Nos. 4,728,455; 5,284,944; 5,246,612; 5,256,779; 5,280,117; 5,274,147; 5,153,161; and 5,227,084.

Cobalt Metal Complexes - Cobalt bleach catalysts useful herein are known, and are described, for example, in U.S. Patent Nos. 5,597,936; 5,595,967; and 5,703,030; and M. L. Tobe, "Base Hydrolysis of Transition-Metal Complexes", Adv. Inorg. Bioinorg. Mech., (1983), 2, pages 1-94. The most preferred cobalt catalyst useful herein are cobalt pentaamine acetate salts having the formula [Co(NH₃)₅OAc] T_y, wherein "OAc" represents an acetate moiety and "T_y" is an anion, and especially cobalt pentaamine acetate chloride, [Co(NH₃)₅OAc]Cl₂; as well as [Co(NH₃)₅OAc](OAc)₂; [Co(NH₃)₅OAc](PF₆)₂; [Co(NH₃)₅OAc](SO₄); [Co(NH₃)₅OAc](BF₄)₂; and [Co(NH₃)₅OAc](NO₃)₂ (herein "PAC").

These cobalt catalysts are readily prepared by known procedures, such as taught for example in U.S. Patent Nos. 5,597,936; 5,595,967; and 5,703,030; in the Tobe article and the references cited therein; and in U.S. Patent 4,810,410; <u>J. Chem. Ed.</u> (1989), <u>66</u> (12), 1043-45; The Synthesis and Characterization of Inorganic Compounds, W.L. Jolly (Prentice-Hall; 1970), pp. 461-3; <u>Inorg. Chem.</u>, <u>18</u>, 1497-1502 (1979); <u>Inorg. Chem.</u>, <u>21</u>, 2881-2885 (1982); <u>Inorg. Chem.</u>, <u>18</u>, 2023-2025 (1979); Inorg. Synthesis, 173-176 (1960); and <u>Journal of Physical Chemistry</u>, <u>56</u>, 22-25 (1952).

Transition Metal Complexes of Macropolycyclic Rigid Ligands - Compositions herein may also suitably include as bleach catalyst a transition metal complex of a macropolycyclic rigid ligand. The phrase "macropolycyclic rigid ligand" is sometimes abbreviated as "MRL" in discussion below. The amount used is a catalytically effective amount, suitably about 1 ppb or more, for example up to about 99.9%, more typically about 0.001 ppm or more, preferably from about 0.05 ppm to about 500 ppm (wherein "ppb" denotes parts per billion by weight and "ppm" denotes parts per million by weight).

Suitable transition metals e.g., Mn are illustrated hereinafter. "Macropolycyclic" means a MRL is both a macrocycle and is polycyclic. "Polycyclic" means at least bicyclic. The term "rigid" as used herein herein includes "having a superstructure" and "cross-bridged". "Rigid" has been defined as the constrained converse of flexibility: see D.H. Busch., Chemical Reviews. (1993), 93, 847-860, incorporated by reference. More particularly, "rigid" as used herein means that the MRL must be determinably more rigid

than a macrocycle ("parent macrocycle") which is otherwise identical (having the same ring size and type and number of atoms in the main ring) but lacking a superstructure (especially linking moieties or, preferably cross-bridging moieties) found in the MRL's. In determining the comparative rigidity of macrocycles with and without superstructures, the practitioner will use the free form (not the metal-bound form) of the macrocycles. Rigidity is well-known to be useful in comparing macrocycles; suitable tools for determining, measuring or comparing rigidity include computational methods (see, for example, Zimmer, Chemical Reviews, (1995), 95(38), 2629-2648 or Hancock et al., Inorganica Chimica Acta, (1989), 164, 73-84.

Preferred MRL's herein are a special type of ultra-rigid ligand which is cross-bridged. A "cross-bridge" is nonlimitingly illustrated in 1.11 hereinbelow. In 1.11, the cross-bridge is a –CH₂CH₂- moiety. It bridges  $N^1$  and  $N^8$  in the illustrative structure. By comparison, a "same-side" bridge, for example if one were to be introduced across  $N^1$  and  $N^{12}$  in 1.11, would not be sufficient to constitute a "cross-bridge" and accordingly would not be preferred.

Suitable metals in the rigid ligand complexes include Mn(II), Mn(III), Mn(IV), Mn(V), Fe(II), Fe(III), Fe(IV), Co(I), Co(II), Co(III), Ni(I), Ni(II), Ni(III), Cu(I), Cu(II), Cu(III), Cr(II), Cr(IV), Cr(V), Cr(VI), V(III), V(IV), V(V), Mo(IV), Mo(V), Mo(VI), W(V), W(VI), Pd(II), Ru(III), Ru(III), and Ru(IV). Preferred transition-metals in the instant transition-metal bleach catalyst include manganese, iron and chromium.

More generally, the MRL's (and the corresponding transition-metal catalysts) herein suitably comprise:

- (a) at least one macrocycle main ring comprising four or more heteroatoms; and
- (b) a covalently connected non-metal superstructure capable of increasing the rigidity of the macrocycle, preferably selected from
- (i) a bridging superstructure, such as a linking moiety;
- (ii) a cross-bridging superstructure, such as a cross-bridging linking moiety; and
- (iii) combinations thereof.

The term "superstructure" is used herein as defined in the literature by Busch et al., see, for example, articles by Busch in "Chemical Reviews".

Preferred superstructures herein not only enhance the rigidity of the parent macrocycle, but also favor folding of the macrocycle so that it co-ordinates to a metal in a cleft. Suitable superstructures can be remarkably simple, for example a linking moiety such as any of those illustrated in Fig. 1 and Fig. 2 below, can be used.



## Fig. 1

wherein n is an integer, for example from 2 to 8, preferably less than 6, typically 2 to 4, or

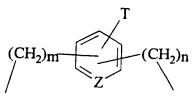


Fig. 2

wherein m and n are integers from about 1 to 8, more preferably from 1 to 3; Z is N or CH; and T is a compatible substituent, for example H, alkyl, trialkylammonium, halogen, nitro, sulfonate, or the like. The aromatic ring in 1.10 can be replaced by a saturated ring, in which the atom in Z connecting into the ring can contain N, O, S or C.

Suitable MRL's are further nonlimitingly illustrated by the following compound:

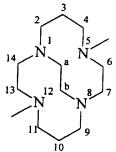


Fig. 3

This is a MRL in accordance with the invention which is a highly preferred, cross-bridged, methyl-substituted (all nitrogen atoms tertiary) derivative of cyclam. Formally, this ligand is named 5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane using the extended von Baeyer system. See "A Guide to IUPAC Nomenclature of Organic Compounds: Recommendations 1993", R. Panico, W.H. Powell and J-C Richer (Eds.), Blackwell Scientific Publications, Boston, 1993; see especially section R-2.4.2.1.

Transition-metal bleach catalysts of Macrocyclic Rigid Ligands which are suitable for use in the invention compositions can in general include known compounds where they conform with the definition herein, as well as, more preferably, any of a large number of novel compounds expressly designed for the present laundry or cleaning uses, and non-limitingly illustrated by any of the following:

Dichloro-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II)

Diaquo-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II)

Hexafluorophosphate

 $\label{lem:approx} A quo-hydroxy-5,12-dimethyl-1,5,8,12-tetra azabicyclo [6.6.2] hexadecane\ Manganese (III)\\ Hexafluorophosphate$ 

Diaquo-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II) Tetrafluoroborate

Dichloro-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(III) Hexafluorophosphate

Dichloro-5,12-di-n-butyl-1,5,8,12-tetraaza bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5,12-dibenzyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-butyl-12-methyl-1,5,8,12-tetraaza- bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-octyl-12-methyl-1,5,8,12-tetraaza- bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-butyl-12-methyl-1,5,8,12-tetraaza- bicyclo[6.6.2]hexadecane Manganese(II).

As a practical matter, and not by way of limitation, the compositions and cleaning processes herein can be adjusted to provide on the order of at least one part per hundred million of the active bleach catalyst species in the aqueous washing medium, and will preferably provide from about 0.01 ppm to about 25 ppm, more preferably from about 0.05 ppm to about 10 ppm, and most preferably from about 0.1 ppm to about 5 ppm, of the bleach catalyst species in the wash liquor. In order to obtain such levels in the wash liquor of an automatic washing process, typical compositions herein will comprise from about 0.0005% to about 0.2%, more preferably from about 0.004% to about 0.08%, of bleach catalyst, especially manganese or cobalt catalysts, by weight of the bleaching compositions.

(d) Other Bleach Catalysts - The compositions herein may comprise one or more other bleach catalysts. Preferred bleach catalysts are zwitterionic bleach catalysts, which are described in U.S. Patent No. 5,576,282 (especially 3-(3,4-dihydroisoquinolinium) propane sulfonate. Other bleach catalysts include cationic bleach catalysts are described in U.S. Patent Nos. 5,360,569, 5,442,066, 5,478,357, 5,370,826, 5,482,515, 5,550,256, and WO 95/13351, WO 95/13352, and WO 95/13353.

Also suitable as bleaching agents are preformed peracids, such as phthalimido-peroxy-caproic acid ("PAP"). See for example U.S. Patent Nos. 5,487,818, 5,310,934, 5,246,620, 5,279,757 and 5,132,431.

Optional Detersive Enzymes - The detergent and cleaning compositions herein may also optionally contain one or more types of detergent enzymes. Such enzymes can include other proteases, amylases, cellulases and lipases. Such materials are known in the art and are commercially available under such trademarks as. They may be incorporated into the non-aqueous liquid detergent compositions herein in the form of suspensions, "marumes" or "prills". Another suitable type of enzyme comprises those in the form of slurries of enzymes in nonionic surfactants, e.g., the enzymes marketed by Novo Nordisk under the tradename "SL" or the microencapsulated enzymes marketed by Novo Nordisk under the tradename "LDP." Suitable enzymes and levels of use are described in U.S. Pat. No. 5,576,282, 5,705,464 and 5,710.115.

Enzymes added to the compositions herein in the form of conventional enzyme prills are especially preferred for use herein. Such prills will generally range in size from about 100 to 1,000 microns, more preferably from about 200 to 800 microns and will be suspended throughout the non-aqueous liquid phase of the composition. Prills in the compositions of the present invention have been found, in comparison with other enzyme forms, to exhibit especially desirable enzyme stability in terms of retention of enzymatic activity over time. Thus, compositions which utilize enzyme prills need not contain conventional enzyme stabilizing such as must frequently be used when enzymes are incorporated into aqueous liquid detergents.

However, enzymes added to the compositions herein may be in the form of granulates, preferably T-granulates.

"Detersive enzyme", as used herein, means any enzyme having a cleaning, stain removing or otherwise beneficial effect in a laundry, hard surface cleaning or personal care detergent composition. Preferred detersive enzymes are hydrolases such as proteases, amylases and lipases. Preferred enzymes for laundry purposes include, but are not limited to, proteases, cellulases, lipases and peroxidases. Highly preferred for automatic dishwashing are amylases and/or proteases, including both current commercially available types and improved types which, though more and more bleach compatible though successive improvements, have a remaining degree of bleach deactivation susceptibility.

Examples of suitable enzymes include, but are not limited to, hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, keratanases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, \(\beta\)-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, and known amylases, or mixtures thereof.

Examples of such suitable enzymes are disclosed in U.S. Patent Nos. 5,705,464, 5,710,115, 5,576,282, 5,728,671 and 5,707,950

The cellulases useful in the present invention include both bacterial or fungal cellulases. Preferably, they will have a pH optimum of between 5 and 12 and a specific activity above 50 CEVU/mg (Cellulose Viscosity Unit). Suitable cellulases are disclosed in U.S. Patent 4,435,307, J61078384 and WO96/02653 which discloses fungal cellulase produced respectively from Humicola insolens, Trichoderma, Thielavia and Sporotrichum. EP 739 982 describes cellulases isolated from novel Bacillus species. Suitable cellulases are also disclosed in GB-A-2.075.028; GB-A-2.095.275; DE-OS-2.247.832 and WO95/26398.

Examples of such cellulases are cellulases produced by a strain of Humicola insolens (Humicola grisea var. thermoidea), particularly the Humicola strain DSM 1800.

Other suitable cellulases are cellulases originated from Humicola insolens having a molecular weight of about 50KDa, an isoelectric point of 5.5 and containing 415 amino acids; and a ~43kD endoglucanase derived from Humicola insolens, DSM 1800, exhibiting cellulase activity; a preferred endoglucanase component has the amino acid sequence disclosed in WO 91/17243. Also suitable cellulases are the EGIII cellulases from Trichoderma longibrachiatum described in WO94/21801 to Genencor. Especially suitable cellulases are the cellulases having color care benefits. Examples of such cellulases are cellulases described in European patent application No. 91202879.2, filed November 6, 1991 (Novo). Carezyme and Celluzyme (Novo Nordisk A/S) are especially useful. See also WO91/17244 and WO91/21801. Other suitable cellulases for fabric care and/or cleaning properties are described in WO96/34092, WO96/17994 and WO95/24471.

Cellulases, when present, are normally incorporated in the cleaning composition at levels from 0.0001% to 2% of pure enzyme by weight of the cleaning composition.

Peroxidase enzymes are used in combination with oxygen sources, e.g. percarbonate, perborate, persulfate, hydrogen peroxide, etc and with a phenolic substrate as bleach enhancing molecule. They are used for "solution bleaching", i.e. to prevent transfer of dyes or pigments removed from substrates during wash operations to other substrates in the wash solution. Peroxidase enzymes are known in the art, and include, for example, horseradish peroxidase, ligninase and haloperoxidase such as chloro- and bromoperoxidase. Suitable peroxidases and peroxidase-containing detergent compositions are disclosed, for example, in U.S. Patent Nos. 5,705,464, 5,710,115, 5,576,282, 5,728,671 and 5,707,950, PCT International Application WO 89/099813, WO89/09813 and in European Patent application EP No. 91202882.6, filed on November 6, 1991 and EP No. 96870013.8, filed February 20, 1996. Also suitable is the laccase enzyme.

Enhancers are generally comprised at a level of from 0.1% to 5% by weight of total composition. Preferred enhancers are substitued phenthiazine and phenoxasine 10-Phenothiazinepropionicacid (PPT), 10-ethylphenothiazine-4-carboxylic acid (EPC), 10-phenoxazinepropionic acid (POP) and 10-methylphenoxazine (described in WO 94/12621) and substitued syringates (C3-C5 substitued alkyl syringates) and phenols. Sodium percarbonate or perborate are preferred sources of hydrogen peroxide.

Said peroxidases are normally incorporated in the cleaning composition at levels from 0.0001% to 2% of pure enzyme by weight of the cleaning composition.

Enzymatic systems may be used as bleaching agents. The hydrogen peroxide may also be present by adding an enzymatic system (i.e. an enzyme and a substrate therefore) which is capable of generating hydrogen peroxide at the beginning or during the washing and/or rinsing process. Such enzymatic systems are disclosed in EP Patent Application 91202655.6 filed October 9, 1991.

Other preferred enzymes that can be included in the cleaning compositions of the present invention include lipases. Suitable lipase enzymes for detergent usage include those produced by microorganisms of the Pseudomonas group, such as Pseudomonas stutzeri ATCC 19.154, as disclosed in British Patent 1,372,034. Suitable lipases include those which show a positive immunological cross-reaction with the antibody of the lipase, produced by the microorganism Pseudomonas fluorescent IAM 1057. This lipase is available from Amano Pharmaceutical Co. Ltd., Nagoya, Japan, under the trade name Lipase P "Amano," hereinafter referred to as "Amano-P". Other suitable commercial lipases include Amano-CES, lipases ex Chromobacter viscosum, e.g. Chromobacter viscosum var. lipolyticum NRRLB 3673 from Toyo Jozo Co., Tagata, Japan; Chromobacter viscosum lipases from U.S. Biochemical Corp., U.S.A. and Disoynth Co., The Netherlands, and lipases ex Pseudomonas gladioli. Especially suitable lipases are lipases such as M1 Lipase^R and Lipomax^R (Gist-Brocades) and Lipolase^R and Lipolase Ultra^R(Novo) which have found to be very effective when used in combination with the compositions of the present invention. Also suitable are the lipolytic enzymes described in EP 258 068, WO 92/05249 and WO 95/22615 by Novo Nordisk and in WO 94/03578, WO 95/35381 and WO 96/00292 by Unilever.

Also suitable are cutinases [EC 3.1.1.50] which can be considered as a special kind of lipase, namely lipases which do not require interfacial activation. Addition of cutinases to cleaning compositions have been described in e.g. WO-A-88/09367 (Genencor); WO 90/09446 (Plant Genetic System) and WO 94/14963 and WO 94/14964 (Unilever).

Lipases and/or cutinases, when present, are normally incorporated in the cleaning composition at levels from 0.0001% to 2% of pure enzyme by weight of the cleaning composition.

In addition to the above referenced lipases, phospholipases may be incorporated into the cleaning compositions of the present invention. Nonlimiting examples of suitable phospholipases included: EC 3.1.1.32 Phospholipase A1; EC 3.1.1.4 Phospholipase A2; EC 3.1.1.5 Lysopholipase; EC 3.1.4.3 Phospholipase C; EC 3.1.4.4. Phospholipase D. Commercially available phospholipases include LECITASE® from Novo Nordisk A/S of Denmark and Phospholipase A2 from Sigma. When phospolipases are included in the compositions of the present invention, it is preferred that amylases are also included. Without desiring to be bound by theory, it is believed that the combined action of the phospholipase and amylase provide substantive stain removal; especially on greasy/oily, starchy and highly colored stains and soils. Preferably, the phospholipase and amylase, when present, are incorporated into the compositions of the present invention at a pure enzyme weight ratio between 4500:1 and 1:5, more preferably between 50:1 and 1:1.

Suitable proteases are the subtilisins which are obtained from particular strains of B. subtilis and B. licheniformis (subtilisin BPN and BPN'). One suitable protease is obtained from a strain of Bacillus, having maximum activity throughout the pH range of 8-12, developed and sold as ESPERASE® by Novo Industries A/S of Denmark, hereinafter "Novo". The preparation of this enzyme and analogous enzymes is described in GB 1,243,784 to Novo. Proteolytic enzymes also encompass modified bacterial serine proteases, such as those described in European Patent Application Serial Number 87 303761.8, filed April 28, 1987 (particularly pages 17, 24 and 98), and which is called herein "Protease B", and in European Patent Application 199,404, Venegas, published October 29, 1986, which refers to a modified bacterial serine protealytic enzyme which is called "Protease A" herein. Suitable is the protease called herein "Protease C", which is a variant of an alkaline serine protease from Bacillus in which Lysine replaced arginine at position 27, tyrosine replaced valine at position 104, serine replaced asparagine at position 123, and alanine replaced threonine at position 274. Protease C is described in EP 90915958:4, corresponding to WO 91/06637, Published May 16, 1991. Genetically modified variants, particularly of Protease C, are also included herein.

A preferred protease referred to as "Protease D" is a carbonyl hydrolase as described in U.S. Patent No. 5,677,272, and WO95/10591. Also suitable is a carbonyl hydrolase variant of the protease described in WO95/10591, having an amino acid sequence derived by replacement of a plurality of amino acid residues replaced in the precursor enzyme corresponding to position +210 in combination with one or more of the following residues: +33, +62, +67, +76, +100, +101, +103, +104, +107, +128, +129, +130, +132, +135, +156, +158, +164, +166, +167, +170, +209, +215, +217, +218, and +222, where the numbered position corresponds to naturally-occurring subtilisin from *Bacillus amyloliquefaciens* or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins, such as *Bacillus lentus* subtilisin (co-pending patent application US Serial No. 60/048,550, filed June 04, 1997 and PCT International Application Serial No. PCT/IB98/00853).

Also suitable for the present invention are proteases described in patent applications EP 251 446 and WO 91/06637, protease BLAP® described in WO91/02792 and their variants described in WO 95/23221.

See also a high pH protease from Bacillus sp. NCIMB 40338 described in WO 93/18140 A to Novo. Enzymatic detergents comprising protease, one or more other enzymes, and a reversible protease inhibitor are described in WO 92/03529 A to Novo. When desired, a protease having decreased adsorption and increased hydrolysis is available as described in WO 95/07791 to Procter & Gamble. A recombinant trypsin-like protease

for detergents suitable herein is described in WO 94/25583 to Novo. Other suitable proteases are described in EP 516 200 by Unilever.

Particularly useful proteases are described in PCT publications: WO 95/30010; WO 95/30011; and WO 95/29979. Suitable proteases are commercially available as ESPERASE[®], ALCALASE[®], DURAZYM[®], SAVINASE[®], EVERLASE[®] and KANNASE[®] all from Novo Nordisk A/S of Denmark, and as MAXATASE[®], MAXACAL[®], PROPERASE[®] and MAXAPEM[®] all from Genencor International (formerly Gist-Brocades of The Netherlands).

Such proteolytic enzymes, when present, are incorporated in the cleaning compositions of the present invention a level of from 0.0001% to 2%, preferably from 0.001% to 0.2%, more preferably from 0.005% to 0.1% pure enzyme by weight of the composition.

Amylases (α and/or β) can be included for removal of carbohydrate-based stains. WO94/02597 describes cleaning compositions which incorporate mutant amylases. See also WO95/10603. Other amylases known for use in cleaning compositions include both α - and β-amylases. α-Amylases are known in the art and include those disclosed in US Pat. no. 5,003,257; EP 252,666; WO/91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent specification no. 1,296,839 (Novo). Other suitable amylases are stability-enhanced amylases described in WO94/18314 and WO96/05295, Genencor, and amylase variants having additional modification in the immediate parent available from Novo Nordisk A/S, disclosed in WO 95/10603. Also suitable are amylases described in EP 277 216.

Examples of commercial  $\alpha$ -amylases products are Purafect Ox Am[®] from Genencor and Termamyl[®], Ban[®], Fungamyl[®] and Duramyl[®], all available from Novo Nordisk A/S Denmark. WO95/26397 describes other suitable amylases :  $\alpha$ -amylases characterised by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by the Phadebas[®]  $\alpha$ -amylase activity assay. Suitable are variants of the above enzymes, described in WO96/23873 (Novo Nordisk). Other amylolytic enzymes with improved properties with respect to the activity level and the combination of thermostability and a higher activity level are described in WO95/35382.

Such amylolytic enzymes, when present, are incorporated in the cleaning compositions of the present invention a level of from 0.0001% to 2%, preferably from 0.00018% to 0.06%, more preferably from 0.00024% to 0.048% pure enzyme by weight of the composition.

The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic

(psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein / genetic engineering techniques in order to optimize their performance efficiency in the laundry detergent and/or fabric care compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant stability, catalytic activity and the like, of the enzyme variant is tailored to suit the particular cleaning application.

In particular, attention should be focused on amino acids sensitive to oxidation in the case of bleach stability and on surface charges for the surfactant compatibility. The isoelectric point of such enzymes may be modified by the substitution of some charged amino acids, e.g. an increase in isoelectric point may help to improve compatibility with anionic surfactants. The stability of the enzymes may be further enhanced by the creation of e.g. additional salt bridges and enforcing calcium binding sites to increase chelant stability.

These optional detersive enzymes, when present, are normally incorporated in the cleaning composition at levels from 0.0001% to 2% of pure enzyme by weight of the cleaning composition. The enzymes can be added as separate single ingredients (prills, granulates, stabilized liquids, etc... containing one enzyme) or as mixtures of two or more enzymes (e.g. cogranulates).

Other suitable detergent ingredients that can be added are enzyme oxidation scavengers. Examples of such enzyme oxidation scavengers are ethoxylated tetraethylene polyamines.

A range of enzyme materials and means for their incorporation into synthetic detergent compositions is also disclosed in WO 9307263 and WO 9307260 to Genencor International, WO 8908694, and U.S. 3,553,139, January 5, 1971 to McCarty et al. Enzymes are further disclosed in U.S. 4,101,457, and in U.S. 4,507,219. Enzyme materials useful for liquid detergent formulations, and their incorporation into such formulations, are disclosed in U.S. 4,261,868.

Enzyme Stabilizers - Enzymes for use in detergents can be stabilized by various techniques. Enzyme stabilization techniques are disclosed and exemplified in U.S. 3,600,319, EP 199,405 and EP 200,586. Enzyme stabilization systems are also described, for example, in U.S. 3,519,570. A useful Bacillus, sp. AC13 giving proteases, xylanases and cellulases, is described in WO 9401532. The enzymes employed herein can be stabilized by the presence of water-soluble sources of calcium and/or magnesium ions in the finished compositions which provide such ions to the enzymes. Suitable enzyme stabilizers and levels of use are described in U.S. Pat. Nos. 5,705,464, 5,710,115 and 5,576,282.

Builders - The detergent and cleaning compositions described herein preferably comprise one or more detergent builders or builder systems. When present, the compositions will typically comprise at least about 1% builder, preferably from about 5%, more preferably from about 10% to about 80%, preferably to about 50%, more preferably to about 30% by weight, of detergent builder. Lower or higher levels of builder, however, are not meant to be excluded.

Preferred builders for use in the detergent and cleaning compositions, particularly dishwashing compositions, described herein include, but are not limited to, water-soluble builder compounds, (for example polycarboxylates) as described in U.S. Patent Nos. 5,695,679, 5,705,464 and 5,710,115. Other suitable polycarboxylates are disclosed in U.S. Patent Nos. 4,144,226, 3,308,067 and 3,723,322. Preferred polycarboxylates are hydroxycarboxylates containing up to three carboxy groups per molecule, more particularly titrates.

Inorganic or P-containing detergent builders include, but are not limited to, the alkali metal, ammonium and alkanolammonium salts of polyphosphates (exemplified by the tripolyphosphates, pyrophosphates, and glassy polymeric meta-phosphates), phosphonates (see, for example, U.S. Patent Nos. 3,159,581; 3,213,030; 3,422,021; 3,400,148 and 3,422,137), phytic acid, silicates, carbonates (including bicarbonates and sesquicarbonates), sulphates, and aluminosilicates.

However, non-phosphate builders are required in some locales. Importantly, the compositions herein function surprisingly well even in the presence of the so-called "weak" builders (as compared with phosphates) such as citrate, or in the so-called "underbuilt" situation that may occur with zeolite or layered silicate builders.

Suitable silicates include the water-soluble sodium silicates with an SiO₂:Na₂O ratio of from about 1.0 to 2.8, with ratios of from about 1.6 to 2.4 being preferred, and about 2.0 ratio being most preferred. The silicates may be in the form of either the anhydrous salt or a hydrated salt. Sodium silicate with an SiO₂:Na₂O ratio of 2.0 is the most preferred. Silicates, when present, are preferably present in the detergent and cleaning compositions described herein at a level of from about 5% to about 50% by weight of the composition, more preferably from about 10% to about 40% by weight.

Partially soluble or insoluble builder compounds, which are suitable for use in the detergent and cleaning compositions, particularly granular detergent compositions, include, but are not limited to, crystalline layered silicates, preferably crystalline layered sodium silicates (partially water-soluble) as described in U.S. Patent No. 4,664,839, and sodium aluminosilicates (water-insoluble). When present in detergent and cleaning compositions, these builders are typically present at a level of from about 1% to 80% by weight,

preferably from about 10% to 70% by weight, most preferably from about 20% to 60% by weight of the composition.

Crystalline layered sodium silicates having the general formula NaMSi_xO_{2x+1}·yH₂O wherein M is sodium or hydrogen, x is a number from about 1.9 to about 4, preferably from about 2 to about 4, most preferably 2, and y is a number from about 0 to about 20, preferably 0 can be used in the compositions described herein. Crystalline layered sodium silicates of this type are disclosed in EP-A-0164514 and methods for their preparation are disclosed in DE-A-3417649 and DE-A-3742043. The most preferred material is delta-Na₂SiO₅, available from Hoechst AG as NaSKS-6 (commonly abbreviated herein as "SKS-6"). Unlike zeolite builders, the Na SKS-6 silicate builder does not contain aluminum. NaSKS-6 has the delta-Na₂SiO₅ morphology form of layered silicate. SKS-6 is a highly preferred layered silicate for use in the compositions described herein herein, but other such layered silicates, such as those having the general formula NaMSi_xO_{2x+1}·yH₂O wherein M is sodium or hydrogen, x is a number from 1.9 to 4, preferably 2, and y is a number from 0 to 20, preferably 0 can be used in the compositions described herein. Various other layered silicates from Hoechst include NaSKS-5, NaSKS-7 and NaSKS-11, as the alpha, beta and gamma forms. As noted above, the delta-Na₂SiO₅ (NaSKS-6 form) is most preferred for use herein. Other silicates may also be useful such as for example magnesium silicate, which can serve as a crispening agent in granular formulations, as a stabilizing agent for oxygen bleaches, and as a component of suds control systems.

The crystalline layered sodium silicate material is preferably present in granular detergent compositions as a particulate in intimate admixture with a solid, water-soluble ionizable material. The solid, water-soluble ionizable material is preferably selected from organic acids, organic and inorganic acid salts and mixtures thereof.

Aluminosilicate builders are of great importance in most currently marketed heavy duty granular detergent compositions, and can also be a significant builder ingredient in liquid detergent formulations. Aluminosilicate builders have the empirical formula:

$$[\mathsf{M}_z(\mathsf{AlO}_2)_y] \cdot \mathsf{xH}_2\mathsf{O}$$

wherein z and y are integers of at least 6, the molar ratio of z to y is in the range from 1.0 to about 0.5, and x is an integer from about 15 to about 264. Preferably, the aluminosilicate builder is an aluminosilicate zeolite having the unit cell formula:

$$Na_z[(AIO_2)_z(SiO_2)_y] \cdot xH_2O$$

wherein z and y are at least 6; the molar ratio of z to y is from 1.0 to 0.5 and x is at least 5, preferably 7.5 to 276, more preferably from 10 to 264. The aluminosilicate builders are preferably in hydrated form and are preferably crystalline, containing from about 10% to about 28%, more preferably from about 18% to about 22% water in bound form.

These aluminosilicate ion exchange materials can be crystalline or amorphous in structure and can be naturally-occurring aluminosilicates or synthetically derived. A method for producing aluminosilicate ion exchange materials is disclosed in U.S. 3,985,669. Preferred synthetic crystalline aluminosilicate ion exchange materials useful herein are available under the designations Zeolite A, Zeolite B, Zeolite P, Zeolite X, Zeolite MAP and Zeolite HS and mixtures thereof. In an especially preferred embodiment, the crystalline aluminosilicate ion exchange material has the formula:

$$Na_{12}[(AlO_2)_{12}(SiO_2)_{12}]\cdot xH_2O$$

wherein x is from about 20 to about 30, especially about 27. This material is known as Zeolite A. Dehydrated zeolites (x = 0 - 10) may also be used herein. Preferably, the aluminosilicate has a particle size of about 0.1-10 microns in diameter. Zeolite X has the formula:

$$Na_{86}[(AlO_2)_{86}(SiO_2)_{106}] \cdot 276H_2O$$

Citrate builders, e.g., citric acid and soluble salts thereof (particularly sodium salt), are polycarboxylate builders of particular importance for heavy duty liquid detergent formulations due to their availability from renewable resources and their biodegradability. Citrates can also be used in granular compositions, especially in combination with zeolite and/or layered silicate builders. Oxydisuccinates are also especially useful in such compositions and combinations.

Also suitable in the detergent compositions described herein are the 3,3-dicarboxy-4-oxa-1,6-hexanedioates and the related compounds disclosed in U.S. 4,566,984. Useful succinic acid builders include the  $C_5$ - $C_{20}$  alkyl and alkenyl succinic acids and salts thereof. A particularly preferred compound of this type is dodecenylsuccinic acid. Specific examples of succinate builders include: laurylsuccinate, myristylsuccinate, palmitylsuccinate, 2-dodecenylsuccinate (preferred), 2-pentadecenylsuccinate, and the like. Laurylsuccinates are the preferred builders of this group, and are described in European Patent Application 86200690.5/0,200,263, published November 5, 1986.

Fatty acids, e.g., C₁₂-C₁₈ monocarboxylic acids, can also be incorporated into the compositions alone, or in combination with the aforesaid builders, especially citrate and/or the succinate builders, to provide additional builder activity. Such use of fatty acids will generally result in a diminution of sudsing, which should be taken into account by the formulator.

<u>Dispersants</u> - One or more suitable polyalkyleneimine dispersants may be incorporated into the cleaning compositions of the present invention. Examples of such suitable dispersants can be found in European Patent Application Nos. 111,965, 111,984, and 112,592; U.S. Patent Nos. 4,597,898, 4,548,744, and 5,565,145. However, any suitable clay/soil

dispersent or anti-redepostion agent can be used in the laundry compositions of the present invention.

In addition, polymeric dispersing agents which include polymeric polycarboxylates and polyethylene glycols, are suitable for use in the present invention. Unsaturated monomeric acids that can be polymerized to form suitable polymeric polycarboxylates include acrylic acid, maleic acid (or maleic anhydride), fumaric acid, itaconic acid, aconitic acid, mesaconic acid, citraconic acid and methylenemalonic acid. Particularly suitable polymeric polycarboxylates can be derived from acrylic acid. Such acrylic acid-based polymers which are useful herein are the water-soluble salts of polymerized acrylic acid. The average molecular weight of such polymers in the acid form preferably ranges from about 2,000 to 10,000, more preferably from about 4,000 to 7,000 and most preferably from about 4,000 to 5,000. Water-soluble salts of such acrylic acid polymers can include, for example, the alkali metal, ammonium and substituted ammonium salts. Soluble polymers of this type are known materials. Use of polyacrylates of this type in detergent compositions has been disclosed, for example, in U.S. 3,308,067.

Acrylic/maleic-based copolymers may also be used as a preferred component of the dispersing/anti-redeposition agent. Such materials include the water-soluble salts of copolymers of acrylic acid and maleic acid. The average molecular weight of such copolymers in the acid form preferably ranges from about 2,000 to 100,000, more preferably from about 5,000 to 75,000, most preferably from about 7,000 to 65,000. The ratio of acrylate to maleate segments in such copolymers will generally range from about 30:1 to about 1:1, more preferably from about 10:1 to 2:1. Water-soluble salts of such acrylic acid/maleic acid copolymers can include, for example, the alkali metal, ammonium and substituted ammonium salts. Soluble acrylate/maleate copolymers of this type are known materials which are described in European Patent Application No. 66915, published December 15, 1982, as well as in EP 193,360, published September 3, 1986, which also describes such polymers comprising hydroxypropylacrylate. Still other useful dispersing agents include the maleic/acrylic/vinyl alcohol terpolymers. Such materials are also disclosed in EP 193,360, including, for example, the 45/45/10 terpolymer of acrylic/maleic/vinyl alcohol.

Another polymeric material which can be included is polyethylene glycol (PEG). PEG can exhibit dispersing agent performance as well as act as a clay soil removal-antiredeposition agent. Typical molecular weight ranges for these purposes range from about 500 to about 100,000, preferably from about 1,000 to about 50,000, more preferably from about 1,500 to about 10,000.

Polyaspartate and polyglutamate dispersing agents may also be used, especially in conjunction with zeolite builders. Dispersing agents such as polyaspartate preferably have a molecular weight (avg.) of about 10,000.

Soil Release Agents - The compositions according to the present invention may optionally comprise one or more soil release agents. If utilized, soil release agents will generally comprise from about 0.01%, preferably from about 0.1%, more preferably from about 0.2% to about 10%, preferably to about 5%, more preferably to about 3% by weight, of the composition. Nonlimiting examples of suitable soil release polymers are disclosed in: U.S. Patent Nos. 5,728,671; 5,691,298; 5,599,782; 5,415,807; 5,182,043; 4,956,447; 4,976,879; 4,968,451; 4,925,577; 4,861,512; 4,877,896; 4,771,730; 4,711,730; 4,721,580; 4,000,093; 3,959,230; and 3,893,929; and European Patent Application 0 219 048.

Further suitable soil release agents are described in U.S. Patent Nos. 4,201,824; 4,240,918; 4,525,524; 4,579,681; 4,220,918; and 4,787,989; EP 279,134 A; EP 457,205 A; and DE 2,335,044.

Chelating Agents - The compositions of the present invention herein may also optionally contain a chelating agent which serves to chelate metal ions and metal impurities which would otherwise tend to deactivate the bleaching agent(s). Useful chelating agents can include amino carboxylates, phosphonates, amino phosphonates, polyfunctionally-substituted aromatic chelating agents and mixtures thereof. Further examples of suitable chelating agents and levels of use are described in U.S. Pat. Nos. 5,705,464, 5,710,115, 5,728,671 and 5,576,282.

The compositions herein may also contain water-soluble methyl glycine diacetic acid (MGDA) salts (or acid form) as a chelant or co-builder useful with, for example, insoluble builders such as zeolites, layered silicates and the like.

If utilized, these chelating agents will generally comprise from about 0.1% to about 15%, more preferably from about 0.1% to about 3.0% by weight of the detergent compositions herein.

Suds suppressor - Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Examples of suitable suds suppressors are disclosed in U.S. Patent Nos. 5,707,950 and 5,728,671. These suds suppressors are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

Softening agents - Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the present invention. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400 898 and in U.S. 5,019,292. Organic softening agents include the water insoluble tertiary amines as disclosed in GB-A-1 514 276 and EP-B-011 340 and their combination with mono C12-C14 quaternary

ammonium salts are disclosed in EP-B-026 527 and EP-B-026 528 and di-long-chain amides as disclosed in EP-B-0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP-A-0 299 575 and 0 313 146.

Particularly suitable fabric softening agents are disclosed in U.S. Patent Nos. 5,707,950 and 5,728,673.

Levels of smectite clay are normally in the range from 2% to 20%, more preferably from 5% to 15% by weight, with the material being added as a dry mixed component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or dilong chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as molten liquid on to other solid components of the composition.

Biodegradable quaternary ammonium compounds as described in EP-A-040 562 and EP-A-239 910 have been presented as alternatives to the traditionally used di-long alkyl chain ammonium chlorides and methyl sulfates.

Non-limiting examples of softener-compatible anions for the quaternary ammonium compounds and amine precursors include chloride or methyl sulfate.

Dye transfer inhibition - The detergent compositions of the present invention can also include compounds for inhibiting dye transfer from one fabric to another of solubilized and suspended dyes encountered during fabric laundering and conditioning operations involving colored fabrics.

Polymeric dye transfer inhibiting agents

The detergent compositions according to the present invention can also comprise from 0.001% to 10 %, preferably from 0.01% to 2%, more preferably from 0.05% to 1% by weight of polymeric dye transfer inhibiting agents. Said polymeric dye transfer inhibiting agents are normally incorporated into detergent compositions in order to inhibit the transfer of dyes from colored fabrics onto fabrics washed therewith. These polymers have the ability to complex or adsorb the fugitive dyes washed out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye transfer inhibiting agents are polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinylpyrrolidone polymers, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. Examples

of such dye transfer inhibiting agents are disclosed in U.S. Patent Nos. 5,707,950 and 5,707,951.

Additional suitable dye transfer inhibiting agents include, but are not limited to, cross-linked polymers. Cross-linked polymers are polymers whose backbone are interconnected to a certain degree; these links can be of chemical or physical nature, possibly with active groups n the backbone or on branches; cross-linked polymers have been described in the Journal of Polymer Science, volume 22, pages 1035-1039.

In one embodiment, the cross-linked polymers are made in such a way that they form a three-dimensional rigid structure, which can entrap dyes in the pores formed by the three-dimensional structure. In another embodiment, the cross-linked polymers entrap the dyes by swelling. Such cross-linked polymers are described in the co-pending European patent application 94870213.9.

Addition of such polymers also enhances the performance of the enzymes according the invention.

pH and Buffering Variation - Many of the detergent and cleaning compositions described herein will be buffered, i.e., they are relatively resistant to pH drop in the presence of acidic soils. However, other compositions herein may have exceptionally low buffering capacity, or may be substantially unbuffered. Techniques for controlling or varying pH at recommended usage levels more generally include the use of not only buffers, but also additional alkalis, acids, pH-jump systems, dual compartment containers, etc., and are well known to those skilled in the art.

The preferred ADD compositions herein comprise a pH-adjusting component selected from water-soluble alkaline inorganic salts and water-soluble organic or inorganic builders as described in U.S. Patent Nos. 5,705,464 and 5,710,115.

Material Care Agents - The preferred ADD compositions may contain one or more material care agents which are effective as corrosion inhibitors and/or anti-tarnish aids as described in U.S. Patent Nos. 5,705,464, 5,710,115 and 5,646,101.

When present, such protecting materials are preferably incorporated at low levels, e.g., from about 0.01% to about 5% of the ADD composition.

Other Materials - Detersive ingredients or adjuncts optionally included in the instant compositions can include one or more materials for assisting or enhancing cleaning performance, treatment of the substrate to be cleaned, or designed to improve the aesthetics of the compositions. Adjuncts which can also be included in compositions of the present invention, at their conventional art-established levels for use (generally, adjunct materials comprise, in total, from about 30% to about 99.9%, preferably from about 70% to about 95%, by weight of the compositions), include other active ingredients such as non-phosphate builders, color speckles, silvercare, anti-tarnish and/or anti-corrosion agents,

dyes, fillers, germicides, alkalinity sources, hydrotropes, anti-oxidants, perfumes, solubilizing agents, carriers, processing aids, pigments, and pH control agents as described in U.S. Patent Nos. 5,705,464, 5,710,115, 5,698,504, 5,695,679, 5,686,014 and 5,646,101. Methods of Cleaning - In addition to the methods for cleaning fabrics, dishes and other hard surfaces, and body parts by personal cleansing, described herein, the invention herein also encompasses a laundering pretreatment process for fabrics which have been soiled or stained comprising directly contacting said stains and/or soils with a highly concentrated form of the cleaning composition set forth above prior to washing such fabrics using conventional aqueous washing solutions. Preferably, the cleaning composition remains in contact with the soil/stain for a period of from about 30 seconds to 24 hours prior to washing the pretreated soiled/stained substrate in conventional manner. More preferably, pretreatment times will range from about 1 to 180 minutes.

The following examples are meant to exemplify compositions of the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention.

In all of the following examples Protease¹ means a protease variant comprising substitution of amino acid residues with another naturally occurring amino acid residue at positions corresponding to positions 101G/103A/104I/159D/232V/236H/245R/248D/252K of *Bacillus amyloliquefaciens* subtilisin. Protease¹ can be substituted with any other additional protease variant of the present invention, with substantially similar results in the following examples.

In the cleaning composition examples of the present invention, the Protease¹ enzyme levels are expressed by pure enzyme by weight of the total composition, the other enzyme levels are expressed by raw material by weight of the total composition, and unless otherwise specified, the other ingredients are expressed by weight of the total composition.

Further, in all of the following examples Amylase³ means an amylase variant according to the present invention.

Further, in the following examples some abbreviations known to those of ordinary skill in the art are used, consistent with the disclosure set forth herein.

Examples 1-7
Liquid Hard Surface Cleaning Compositions

				Exam	ple No.		
Component	1	2	3	4	5	_ 6	7
Protease ¹	0.05	0.05	0.20	0.02	0.03	0.10	
Protease ²	-	. <del>-</del>	_	_	0.05		0.03
Amylase ³	-	0.002	0.002	0.0005	0.04	0.20 0.0008	0.1
Chelant**	-	_	_	2.90	2.90	0.0006	0.005
Citrate	-	_	_		2.90	-	_
LAS	-	1.95	_	1.95	_	2.90	2.90
AS	2.00	_	2.20	_	2.20	1.95	-
AES	2.00	_	2.20	_	2.20	_	2.20
Amine Oxide	0.40		0.50			_	2.20
Hydrotrope	_	1.30	0.50	1.20	0.50	-	0.50
Solvent***	_		-	1.30	-	1.30	-
Water and Minors	-	6.30	6.30	6.30	6.30	6.30	6.30
water and wimors				balance to	0 100%		

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

In Examples 6 and 7, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

^{**}Na₄ ethylenediamine diacetic acid

^{***}Diethyleneglycol monohexyl ether

^{****}All formulas adjusted to pH 7

Examples 2-7

<u>Dishwashing Composition</u>

	Example No.						
Component	2	3	44	5	6	7	
Protease ¹	0.05	0.50	0.02	0.40	0.10	0.03	
Protease ²	-	-	-	-	0.40	0.1	
Amylase ³	0.002	0.002	0.0005	0.04	0.0008	0.005	
TFAA I	0.90	0.90	0.90	0.90	0.90	0.90	
AES	12.00	12.00	12.00	12.00	12.00	12.00	
2-methyl undecanoic acid	4.50	4.50		4.50	4.50	~~	
C ₁₂ ethoxy (2) carboxylate	4.50	4.50	4.50	4.50°	4.50	4.50	
C ₁₂ alcohol ethoxylate (4)	3.00	3.00	3.00	3.00	3.00	3.00	
Amine oxide	3.00	3.00	3.00	3.00	3.00	3.00	
Hydrotrope	2.00	2.00	2.00	2.00	2.00	2.00	
Ethanol	4.00	4.00	4.00	4.00	4.00	4.00	
$Mg^{++}$ (as $MgCl_2$ )	0.20	0.20	0.20	0.20	0.20	0.20	
Ca ⁺⁺ (as CaCl ₂ )	0.40	0.40	0.40	0.40	0.40	0.40	
Water and Minors****			balance 1	to 100%			

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

### **** Product pH is adjusted to 7.

In Examples 6 and 7, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

Example 8

Dishwashing Compositions (A&B ADW; C Liquid)

A	В	С
17.5	-	-
	15.0	_
0.80	-	-
-	5.10	_
8.30	-	_
-	8.50	-
3.99	-	_
2.00	-	_
	0.80 - 8.30 - 3.99	17.5 - 15.0 0.80 5.10 8.30 8.50 3.99 -

3.2r Na Silicate	£ 10		
Aluminum tristearate	5.18	-	-
Nonionic surfactant	0.10	-	-
NaAE0.6S	-	2.50	-
Glucose amide	-	-	24.70
C10E8	-	-	3.09
Betaine	-	-	4.11
Amine oxide	-	-	2.06
Magnesium as oxide	-	-	2.06
Hydrotrope	-	-	0.49
Sodium hypochlorite as AvCl ₂	-	-	4.47
Amylase ³	1.15	-	-
Protease ¹	0.002	0.03	0.005
Balance to 100%	0.01	0.43	0.05

Example 9

Liquid Dishwashing Compositions (especial	ly suitable unde	I Japanese condit	ions)
Component	A	B	TOIIS
AE1.4S	24.69		
N-cocoyl N-methyl glucamine		24.69	
Amine oxide	3.09	3.09	
Betaine	2.06	2.06	
Nonionic surfactant	2.06	2.06	
	4.11	4.11	
Hydrotrope	4.47	4.47	
Magnesium	0.49	0.49	
Ethanol	7.2	7.2	
LemonEase	0.45	_	
Geraniol/BHT	0.43	0.45	
Amylase ³	-	0.60/0.02	
Protease ¹	0.03	0.005	
	0.01	0.43	
Balance to 100%			

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Example 10
Granular Automatic Dishwashing Composition

Commonant		ZIII OII	
Component	A	В	<u>C</u>
Citric Acid	15.0	-	-
Citrate	4.0	29.0	15.0
Acrylate/methacrylate copolymer	6.0	-	6.0
Acrylic acid maleic acid copolymer	-	3.7	-
Dry add carbonate	9.0	_	20.0
Alkali metal silicate	8.5	17.0	9.0
Paraffin	-	0.5	
Benzotriazole	-	0.3	_
Amylase ³	1.6	1.6	1.6
Protease ¹	0.2	0.1	0.06
Percarbonate (AvO)	1.5	-	0.00
Perborate monohydrate	-	0.3	1.5
Perborate tetrahydrate	-	0.9	
Tetraacetylethylene diamine	3.8	4.4	
Diethylene triamine penta methyl phosphonic acid	0.13	0.13	0.13
(Mg salt)		31.2	0.15
Alkyl ethoxy sulphate - 3 times ethoxylated	3.0	_	_
Alkyl ethoxy propoxy nonionic surfactant	-	1.5	_
Suds suppressor	2.0	•	-
Olin SLF18 nonionic surfactant	-	_	2.0
Sulphate	Balance to	0 100%	2.0

Example 11

Compact high density (0.96Kg/l) dishwashing detergent compositions A to F in accordance with the invention:

Component	A	В	С	D	Е	F
STPP	-	51.4	51.4	-	1-	44.3
Citrate	17.05	-	-	49.6	40.2	5
Carbonate	17.50	14.0	20.0		8.0	33.6
Bicarbonate		-	-	26.0	1-	-
Silicate	14.81	15.0	8.0	-	25.0	3.6
Metasilicate	2.50	4.5	4.5	-	-	-
PB1	9.74	7.79	7.79	_	-	
PB4	-		_	9.6		1_

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Percarbonate	-	_				- 1
Nonionic	2.00	1.50			11.8	4.8
TAED	2.39		1.50	2.6	1.9	5.9
HEDP	1.00	<del>-  </del>	<del></del>	3.8	-   -	1.4
DETPMP			- <del>  -</del>	<del>- </del>		_
Mn TACN	0.65	<del></del>	<del>-  </del>			
PAAC	<del>-  </del>	<del>-   -</del>	<u> </u>		0.008	-
	<del>-</del>	0.008	0.008	_   -	_	
Paraffin	0.50	0.38	0.38	0.6		<del></del>
Protease ¹	0.1	0.06	0.05	0.03		+
Amylase ³	1.5	1.5	1.5		0.07	0.01
BTA	0.30	0.22		2.6	2.1	0.8
Polycarboxylate	6.0		0.22	0.3	0.3	0.3
Perfume		-	<del>-</del>	<del> </del>	4.2	0.9
	0.2	0.12	0.12	0.2	0.2	0.2
Sulphate / Water	20.57	1.97	2.97	3.6	4.5	3.9
pH (1% solution)	11.0	11.0	11.3	9.6	10.8	10.9

Example 12

Granular dishwashing detergent compositions examples A to F of bulk density

1.02Kg/L in accordance with the invention:

Component	Α	В	С	D		T_
STPP	30.00	33.5	27.9		E	F
Carbonate	30.50	30.50	30.5	29.62	33.8	22.0
Silicate	7.40	7.50	12.6	23.00	34.5	45.0
Metasilicate		4.5	12.0	13.3	3.2	6.2
Percarbonate	_		<del> </del>		+	
PB1	4.4	4.5	4.3		4.0	
NaDCC		1.5	4.5		<del> </del>	
Nonionic	1.0	0.75	10	2.00	<del> -</del>	0.9
TAED	1.00	- 0.73	1.0	1.90	0.7	0.5
PAAC	- 1.00	0.004	<del> </del>	<del> </del>	0.9	-
Paraffin	0.25	0.004	<del> </del>	+	<del> -</del>	<del></del>
Protease ¹	0.05	0.23	0.005	<del> -</del>	<u> -</u>	<del> </del>
Amylase ³	0.38		0.025	0.1	0.02	0.07
ВТА	0.15	0.64	0.46		0.6	ļ
Perfume		0.15	<del> </del>	<del> -</del>	0.2	
Sulphate/water	0.2	0.2	0.05	0.1	0.2	
raiphate/water	23.45	16.87	22.26	30.08	21.7	25.4

pH (1% solution)	10.80	11.3	11.0	10.70	11.5	10.9

Example 13

Tablet detergent composition examples A to H in accordance with the present invention are prepared by compression of a granular dishwashing detergent composition at

a pressure of 13KN/cm² using a standard 12 head rotary press:

Component		1					1	<del></del>
	<u> </u>	В	C	D	E	F	G	H
STPP	<del> -</del>	48.8	54.7	38.2	-	52.4	56.1	36.0
Citrate	20.0	<u> </u>	-	<u> </u>	35.9		_   -	-
Carbonate	20.0	5.0	14.0	15.4	8.0	23.0	20.0	28.0
Silicate	15.0	14.8	15.0	12.6	23.4	2.9	4.3	4.2
Protease ¹	0.05	0.09	0.05	0.03	0.06	0.03	0.03	0.1
Amylase ³	1.5	1.5	1.5	0.85	1.9	0.4	2.1	0.3
PB1	14.3	7.8	11.7	12.2	-	-	6.7	8.5
PB4	-	_	-	_	22.8	_	3.4	-
Percarbonate	-	-	-	-	-	10.4	-	-
Nonionic	1.5	2.0	2.0	2.2	1.0	4.2	4.0	6.5
PAAC	<u>  -                                   </u>		0.016	0.009	_	-	-	-
MnTACN	<u>  -                                   </u>	_	-	_	0.007	-	_	_
TAED	2.7	2.4	-	_	-	2.1	0.7	1.6
HEDP	1.0	-	-	0.93	-	0.4	0.2	
DETPMP	0.7	_	-		-	-	-	-
Paraffin	0.4	0.5	0.5	0.55	-	-	0.5	-
BTA	0.2	0.3	0.3	0.33	0.3	0.3	0.3	-
Polycarboxylate	4.0	_	•	-	4.9	0.6	0.8	_
PEG	-	-	-	_	-	2.0	-	2.0
Glycerol	<u>.</u> ·	-	-	-	-	0.4	_	0.5
Perfume	_	-	•	0.05	0.20	0.2	0.2	0.2
Sulphate / water	17.4	14.7	_	15.74		_	-	11.3
weight of tablet	20g	25g	20g	30g	18g	20g	25g	24.0
pH (1% solution)	10.7	10.6	10.7	10.7	10.9	11.2	11.0	10.8

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Example 14

Dimple Tablet Automatic Dishwashing Composition

	<u> Dimple Tablet Automatic Dishwashi</u>	ng Composition	
Component	A (% R.M.)	B (g R.M.)	C (- D ) ()
Tablet Body	,	22 (8 14.171.)	$\underline{C}$ (g R.M.)
Sodium Carbonate	15.348	3.500	
STPP (12% $H_2O$ )	46.482	10.600	5.25
Gran HEDP	0.789		9.93
SKS 6	6.578	0.180	0.28
2 ratio Silicate	7.016	1.500	2.25
PB1		1.600	1.65
Termamyl 2x PCA	10.743	2.450	3.68
Savinase	0.491	0.112	.17
Plurafac	0.526	0.120	0.18
BTA	3.508	0.800	0.9
PEG	0.263	0.060	0.09
PEG 4000	1.140	0.260	-
_	-	-	0.39
Winog	0.439	0.100	0.15
Perfume	0.101	0.023	0.01
Dimple Filling			0.01
Citric Acid	0.987	0.225	0.23
Bicarbonate	2.600	0.593	0.59
Sandolan EHRL Dye	0.007	0.0017	
PEG 400/4000	0.395	0.090	0.0017
PEG 400	-	-	0.00
PEG 4000	•	-	0.02
Protease ¹	0.05	0.040	0.08
Amylase ³	1.412	0.268	0.27
	1.412	0.322	0.32

## Granular Fabric Cleaning Composition

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more protease enzymes, preferably from about 0.001% to about 10%, more preferably, from about 0.005% to about 5%, more preferably from 0.01% to about 1% by weight of active protease enzyme of the composition. (See U.S. Patent No. 5,679,630 Examples).

Example 15
Granular Fabric Cleaning Composition

Example No. Component В C D Protease¹ 0.10 0.20 0.03 0.05 Protease² 0.2 0.15 Amylase³ 0.002 0.0005 0.04 0.005 C₁₃ linear alkyl benzene sulfonate 22.00 22.00 22.00 22.00 Phosphate (as sodium 23.00 23.00 23.00 23.00 tripolyphosphates) Sodium carbonate 23.00 23.00 23.00 23.00 Sodium silicate 14.00 14.00 14.00 14.00 Zeolite 8.20 8.20 8.20 8.20 Chelant (diethylaenetriamine-0.40 0.40 0.40 0.40 pentaacetic acid) Sodium sulfate 5.50 5.50 5.50 5.50 Water balance to 100%

In Examples 15 C and D, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

Example 16
Granular Fabric Cleaning Composition

		Exam	ple No.	
Component	A	B	_ C	D
Protease ¹	0.10	0.20	0.03	0.05
Protease ²	-	-	0.2	0.03
Amylase ³	0.005	0.0005	0.04	0.002
C ₁₂ alkyl benzene sulfonate	12.00	12.00	12.00	12.00

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

1	1	_
	1	υ

2-butyl octanoic acid C ₁₂ -C ₁₄ secondary (2,3) alkyl sulfate, Na salt	4.00 5.00	4.00 5.00	4.00 5.00	4.00 5.00
Sodium citrate	5.00	<i>5</i> 00	_	
Optical brightener	5.00	5.00	5.00	5.00
	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	
Fillers, water, minors	17.00		17.00	17.00
² Drotocco other days		balance	e to 100%	

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

In Examples 16 C and D, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

Example 17
Granular Fabric Cleaning Compositions

Samular 1 de	The Cleaning Composition	S
Components	Example N	<u>lo.</u>
Linear alkyl homes at t	А	B
Linear alkyl benzene sulphonate	11.4	10.70
Tallow alkyl sulphate	1.80	2.40
C ₁₄₋₁₅ alkyl sulphate	3.00	
C ₁₄₋₁₅ alcohol 7 times ethoxylated		3.10
·	4.00	4.00
Tallow alcohol 11 times ethoxylated	1.80	1.80
Dispersant	0.07	0.1
Silicone fluid	0.80	0.80
Trisodium citrate		
Citric acid	14.00	15.00
Zeolite	3.00	2.50
	32.50	32.10
Maleic acid acrylic acid copolymer	5.00	5.00
Diethylene triamine penta methylene		
phosphonic acid	1.00	0.20

Protease ¹	0.1	0.01
Lipase	0.36	0.40
Amylase ³	0.30	0.30
Sodium silicate	2.00	2.50
Sodium sulphate	3.50	5.20
Polyvinyl pyrrolidone	0.30	0.50
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.1	0.1
Minors	Up to 100	Up to 100

Example 18
Granular Fabric Cleaning Compositions

	Examp	ole No.
Components	A	В
Sodium linear C ₁₂ alkyl benzene-sulfonate	6.5	8.0
Sodium sulfate	15.0	18.0
Zeolite A	26.0	22.0
Sodium nitrilotriacetate	5.0	5.0
Polyvinyl pyrrolidone	0.5	0.7
Tetraacetylethylene diamine	3.0	3.0
Boric acid	4.0	-
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Protease ¹	0.02	0.05
Amylase ³	0.005	0.002
Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

# Example 19 Compact Granular Fabric Cleaning Composition

Components	
Alkyl Sulphate	Weight %
Alkyl Ethoxy Sulphate	8.0
Mixture of C25 and C45 alcohol 3 and 7 times ethoxylated	2.0
Polyhydroxy fatty acid amide	6.0
Zeolite	2.5
Layered silicate/citrate	17.0
Carbonate	16.0
1.12	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Protease ¹	0.03
Lipase	0.2
Cellulase	0.2
Amylase ³	_
Tetracetylethylene diamine	0.005
Percarbonate	6.0
Ethylene diamine disuccinic acid	22.0
Suds suppressor	0.3
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-	3.5
ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyril) biphenyl	
Water, Perfume and Minors	0.05
U	p to 100

# Example 20 Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C ₁₆ -C ₁₈ alkyl sulfate	1.3
C ₁₄₋₁₅ alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	
Maleic acid acrylic acid copolymer	15.0
Diethylene triamine penta methylene phosphonic acid	4.0
Perborate	0.4
Tetraacetylethylene diamine	15.0
Smectite clay	5.0
Poly (oxy ethylene) (MW 300,000)	10.0
Protease ¹	0.3
Lipase	0.02
Amylase ³	0.2
•	0.3
Cellulase	0.2
Sodium silicate	3.0
Sodium carbonate	10.0
Carboxymethyl cellulose	0.2
Brighteners	0.2
Water, perfume and minors	Up to 100

## Example 21

## Granular Fabric Cleaning Composition

Component	Composition
Linear alkyl benzene sulfonate	Weight %
Tallow alkyl sulfate	6.92
C ₁₄₋₁₅ alcohol 7 times ethoxylated	2.05
C ₁₂₋₁₅ alkyl ethoxy sulfate - 3 times ethoxylate	4.4
Zeolite	0.16
Citrate	20.2
Carbonate	5.5
Silicate	15.4
Maleic acid acrylic acid copolymer	3.0
Carboxymethyl cellulase	4.0
Soil release polymer	0.31
Protease ¹	0.30
Lipase	0.1
Cellulase	0.36
Amylase ³	0.13
Perborate tetrahydrate	0.005
Perborate monohydrate	11.64
Tetraacetylethylene diamine	8.7
	5.0
Diethylene tramine penta methyl phosphonic acid Magnesium sulfate	0.38
Brightener	0.40
Perfume, silicone, suds suppressors	0.19
Minors	0.85
	Up to 100

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Example 22
Granular Fabric Cleaning Composition

Component Component	ric Cleaning Compositio		
Base Granule Components	A	<u>B</u>	<u>C</u>
LAS/AS/AES (65/35)	9.95		
LAS/AS/AES (70/30)		-	-
Alumino silicate	- 14.06	12.05	7.70
Sodium carbonate	11.86	15.74	17.10
Sodium silicate	0.58	12.74	13.07
NaPAA Solids	2.26	0.58	0.58
PEG Solids	1.01	2.26	1.47
Brighteners	0.17	1.12	0.66
DTPA	0.17	0.17	0.11
Sulfate	5 46	•	0.70
DC-1400 Deaerant	5.46	6.64	4.25
Moisture	0.02	0.02	0.02
Minors	3.73	3.98	4.33
B.O.T. Spray-on	0.31	0.49	0.31
Nonionic surfactant	0.50	0.50	
Agglomerate Components	0.30	0.50	0.50
LAS/AS (25/75)	11.70	0.60	
Alumino silicate	13.73	9.60	10.47
Carbonate	8.11	11.26 6.66	12.28
PEG 4000	0.59	0.48	7.26
Moisture/Minors	4.88	4.00	0.52
Functional Additives	4.00	4.00	4.36
Sodium carbonate	7.37	6.98	7.45
Perborate	1.03	1.03	7.45
AC Base Coating	-	1.00	2.56
NOBS	-	1.00	-
Soil release polymer	0.41	0.41	2.40
Cellulase	0.33	0.33	0.31
Protease ¹	0.1	0.05	0.24
Amylase ³	0.002	0.03	0.15
AE-Flake	0.40	0.005	0.04
Liquid Spray-on	V.7U	V.4U	0.29
Perfume	0.42	0.42	0.40
	V.74	U.42	0.42

Noionic spray-on Minors

1.00

1.00

0.50

Up to 100

Example 23
Granular Fabric Cleaning Composition

	A	В
Surfactant		
- Na LAS	6.40	-
- KLAS	-	9.90
- AS/AE3S	6.40	4.39
- TAS	0.08	0.11
- C24AE5	3.48	0.11
- Genagen	_	1.88
- N-cocoyl N-methyl	1.14	_
glucamine (lin)		2.82
- C ₈₋₁₀ dimethyl	1.00	1.40
hydroxyethyl		1.40
ammonium chloride		
Builder		
- Zeolite	20.59	13.39
- SKS-6	10.84	ì
- Citric Acid	2.00	10.78
Buffer	2.00	-
- Carbonate	9.60	10.05
- Bicarbonate	2.00	12.07
- Sulphate	2.64	2.00
- Silicate		-
olymer	0.61	0.16
- Acrylic acid/maleic		
	1.17	1.12
cid copolymer (Na) - CMC		
- CHAC	0.45	0.24

	123	
- Polymer	0.34	0.18
- Hexamethylene-	1.00	1.00
diamine tetra-E24		
ethoxylate,		
diquaternized with		
methyl chloride		
Enzyme		
- Protease ⁱ	0.03	0.03
(% pure enzyme)	: 1	
- Cellulase	0.26	0.26
- Amylase ³	0.65	0.73
~ Lipase	0.27	0.15
Bleach		
- TAED (100%)	3.85	3.50
- Phenoisulfonate	-	2.75
ester of N-nonanoyl-6-		
aminocaproie acid		
- Percarbonate	16.20	18.30
- HEDP	0.48	0.48
- EDDS	0.30	0.30
Miscellaneous		
- Malic particle		2.20 + bicarb
- Brightener 15/49	0.077/0.014	0.07/0.014
- Zine phthalocyanine	0.0026	0.0026
sulfonate		
- Polydimethylsiloxane	0.25	0.24
with trimethylsilyl end		
blocking units		
- Soap	-	1.00
- Perfume	0.45	0.55
TOTAL	100	100

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Example 24
Granular Fabric Cleaning Composition

	A	В
	%	%
Surfactant		
NaLAS	6.8	0.4
KLAS	_	10.9
FAS	0.9	0.1
AS	0.6	1.5
C25AE3S	0.1	1.5
AE5	4.2	-
N-Cocoyl-N-Methyl Glucamine	-	1.0
Genagen	_	1.8
C ₈₋₁₀ dimethyl hydroxyethyl	_	1.2
ammonium chloride	-	1.0
Builder		
SKS-6	3.3	
Zeolite	17.2	9.0
Citric Acid	1.5	18.9
Buffer	1.5	
Carbonate	21.1	
Sodium Bicarbonate	21.1	15.0
Sulphate	15.2	2.6
Malic Acid	13.2	5.5
Silicate		2.9
olymer	0.1	-
acrylic acid/maleic acid copolymer	2.2	
Na)	2.2	0.9
examethylene-diamine tetra-E24	0.5	
choxylate, diquaternized with	0.5	0.7
ethyl chloride		
olymer		
MC	0.1	0.1
nzymes	0.2	0.1
*		
otease ¹ (% pure enzyme)	0.02	0.05
pase	0.18	0.14

Amylase ³	1 0.4	1
Cellulase	0.64	0.73
	0.13	0.26
Bleach		
TAED	2.2	2.5
Phenolsulfonate ester of N-nonanoyl-	-	1.96
6-aminocaproic acid		1.50
Sodium Percarbonate	-	13.1
PB4	15.6	_
EDDS	0.17	0.21
MgSO4	0.35	0.47
HEDP	0.15	0.34
Miscellaneous		
Brightener	0.06	0.04
- Zinc phthalocyanine sulfonate	0.0015	0.0020
- Polydimethylsiloxane with	0.04	0.14
trimethylsilyl end blocking units		
Soap	0.5	0.7
Perfume	0.35	0.45
Speckle	0.5	0.6

Examples 25
Granular laundry detergent compositions 25 A-C are of particular utility under European machine wash conditions and are prepared in accordance with the invention:

Component	A	В	С
LAS	7.0	5.61	4.76
TAS	-	-	1.57
C45AS	6.0	2.24	3.89
C25E3S	1.0	0.76	1.18
C45E7		-	2.0
C25E3	4.0	5.5	-

QAS	0.8	2.0	2.0
STPP		-	
Zeolite A	25.0	19.5	19.5
Citric acid	2.0	2.0	2.0
NaSKS-6	8.0	10.6	10.6
Carbonate I	8.0	10.0	8.6
MA/AA	1.0	2.6	1.6
СМС	0.5	0.4	0.4
PB4	-	12.7	-
Percarbonate	-	-	19.7
TAED		3.1	5.0
Citrate	7.0	-	-
DTPMP	0.25	0.2	0.2
HEDP	0.3	0.3	0.3
QEA 1	0.9	1.2	1.0
Protease ¹	0.02	0.05	0.035
ipase	0.15	0.25	0.15
Cellulase	0.28	0.28	0.28
amylase	0.4	0.7	0.3
VPI/ PVNO	0.4	-	0.1
hotoactivated bleach (ppm)	15 ppm	27 ppm	27 ppm

Brightener 1	0.08	0.19	0.19
Brightener 2	•	0.04	0.04
Perfume	0.3	0.3	0.3
Effervescent granules (malic acid 40%, sodium bicarbonate 40%, sodium carbonate 20%)	15	15	5
Silicone antifoam	0.5	2.4	2.4
Minors/inerts to 100%			

Example 26

The following formulations are examples of compositions in accordance with the invention, which may be in the form of granules or in the form of a tablet.

Component	26
Base Product	
C45 AS/TAS	3.0
LAS	8.0
C25AE3S	1.0
NaSKS-6	9.0
C25AE5/AE3	5.0
Zeolite A	10.0
SKS-6 (I) (dry add)	2.0
MA/AA	2.0
Citric acid	1.5
EDDS	0.5
HEDP	0.2
PB1	10.0
NACA OBS	2.0
TAED	2.0
Carbonate	8.0
Sulphate	2.0
Amylase ³	0.3
Lipase	0.2
Protease ¹	0.02
Minors (Brightener/SRP1/	0.5
CMC/Photobleach/ MgSO4/	
PVPVI/Suds suppressor/	
PEG)	

Perfume	0.5
1 Ci i dilic	1 0.5

Example 27

The following granular laundry detergent compositions 27 A-E are of particular utility under Japanese machine wash conditions and are prepared in accord with the invention:

Component	A	В	С	D	E
LAS	23.57	23.57	21.67	21.68	21.68
FAS	4.16	4.16	3.83	3.83	3.83
Nonionic surfactant	3.30	3.30	2.94	3.27	3.27
Bis (hydroxyethyl) methyl alkyl ammonium chloride	0.47	0.47	1.20	1.20	1.20
SKS-6	7.50	7.50	5.17	5.76	5.06
Polyacrylate copolymer (MW 11000) (maleic/acrylate ratio of 4:6)	7.03	7.03	14.36	14.36	14.36
Zeolite	11.90	11.40	10.69	11.34	11.34
Carbonate	14.90	14.82	11.71	11.18	11.18
Silicate	12.00	12.00	12.37	12.38	12.38
Protease ¹	0.016	0.016	0.046	0.046	0.046
Lipase	-	-	0.28	-	-
Amylase ²	-	•	0.62	•	-
Cellulase	-	-	0.48	<u>-</u>	0.70
NOBS	3.75	3.75	2.70	2.70	2.70
PB1	3.53	_	2.60	-	-

Sodium percarbonate	-	4.21	•	3.16	3.16
SRP	0.52	0.52	0.70	0.70	0.70
Brightener	0.31	0.31	0.28	0.28	0.50
AE-coflake	0.17	0.20	0.17	0.17	0.17
Polydimethylsiloxane	-	-	0.68	0.68	0.68
Perfume	0.06	0.06	0.08	-	-
Perfume	-	•	<u>.</u>	0.23	0.23
Hydrophobic precipitated silica	0.30	0.30	0.30	0.30	0.30
PEG4000	0.19	0.19	0.17	0.17	0.17
Minors/inerts to 100%					

#### Liquid Fabric Cleaning Compositions

Liquid fabric cleaning compositions of the present invention preferably comprise an effective amount of one or more protease enzymes, preferably from about 0.0001% to about 10%, more preferably from about 0.001% to about 1%, and most preferably from about 0.001% to about 0.1% by weight of active protease enzyme of the composition. (See U.S. Patent No. 5,679,630 Examples).

Example 28

<u>Liquid Fabric Cleaning Compositions</u>

Example No. Component В  $\mathbf{C}$ D E Protease¹ 0.05 0.03 0.30 0.03 0.10 Protease² 0.1 0.20 Amylase³ C₁₂- C₁₄ alkyl sulfate, Na 20.00 20.00 20.00 20.00 20.00 2-Butyl octanoic acid 5.00 5.00 5.00 5.00 5.00 Sodium citrate 1.00 1.00 1.00 1.00 1.00 C₁₀ alcohol ethoxylate (3) 13.00 13.00 13.00 13.00 13.00 Monethanolamine 2.50 2.50 2.50 2.50 2.50 Water/propylene glycol/ethanol (100:1:1) balance to 100%

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

In Examples 28 D and E, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

Examples 29
Liquid Fabric Cleaning Compositions

	Examp	ple No.
Component	A	<u>B</u>
C ₁₂₋₁₄ alkenyl succinic acid	3.0	8.0
Citric acid monohydrate	10.0	15.0
Sodium C ₁₂₋₁₅ alkyl sulphate	8.0	8.0
Sodium sulfate of C ₁₂₋₁₅ alcohol 2 times ethoxylated	-	3.0
C ₁₂₋₁₅ alcohol 7 times ethoxylated	-	8.0
C ₁₂₋₁₅ alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta (methylene phosphonic acid)	0.2	
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Protease ¹	0.01	0.02
Amylase ³	0.005	0.002
Polyvinyl pyrrolidone	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to p	H 7.5
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.4	0.1
Waters and minors		100 %

Example 30
Liquid Fabric Cleaning Compositions

	Example No.
Component	28
NaLAS (100%am)	16
Neodol	21.5
Citrate	6.8
EDDS	
Dispersant	1.2
Perborate	1.3
Phenolsulfonate ester of N-nonanoyl-6-aminocaproic acid	12
Protease ¹ (% pure enzyme)	. 6
Amylase ³	0.03
	0.40
Carezyme	0.03
Solvent (BPP)	18.5
Polymer	0.1
Carbonate	10
FWA 15	0.2
TiO ₂	
PEG 8000	0.5
Perfume	0.4
	1.0-1.2
Suds suppressor	0.06
Waters and minors	up to 100%

Examples 31
Liquid Fabric Cleaning Compositions

	<u>Exam</u>	ple No.
Component	Α	В
Dl H₂O	38.63	-
MEA	0.48	9.0
NaOH	4.40	1.0
Pdiol	4.00	10.0
Citric acid	2.50	2.0

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DTPA	0.50	
FWA Premix (Br 15/MEA/NI 23-9)		1.0
Na C25AE1.80S	0.15	0.15
	23.50	-
AE3S (H)	•	4.0
C11.8HLAS	3.00	
Neodol	2.00	14.0
EtOH		6.0
Ca*Formate	0.50	2.0
	0.10	0.1
Borax premix (Borax/MEA/Pdiol/CitricAcid)	2.50	-
C10 APA	1.50	_
TEPA 105	1.20	_
FA C12-18	5.00	-
Neptune LC		-
Dye	0.50	-
Cellulase	0.0040	0.0015
	0.053	0.2
Amylase ³	0.15	0.2
Protease ¹	0.1	0.1
DC 2-3597	0.12	
Rapeseed FA		0.2
Waters and minors	6.50	4.0
word and infliors	up to 100 %	6

Example 32
Liquid Fabric Cleaning Composition

Component	<u>30</u>
NaOH	5.50
Pdiol	
Citric acid	6.90
DTPA	1.50
FWA Premix (Br 15/MEA/NI 23-9)	1.50
·	0.15
AE3S (H)	2.50
LAS (H)	13.0
Neodol	
EtOH	2.00
	3.50
Ca*Formate	0.10
Boric acid	1.00
Clay	
<del>-</del>	4.00

Amylase ³	0.15
Protease ¹	0.02
Fatty Acid	16.50
Waters and minors	up to 100 %

Example 34
Liquid Fabric Cleaning Composition

The following liquid fabric cleaning composition of particular utility under Japanese machine wash conditions is prepared in accordance with the invention:

Component	34
AE2.5S	15.00
AS	5.50
N-Cocoyl N-methyl glucamine	5.00
Nonionic surfactant	4.50
Citric acid	3.00
Fatty acid	5.00
Base	0.97
Monoethanolamine	5.10
1,2-Propanediol	7.44
EtOH	5.50
HXS	1.90
Boric acid	3.50
Ethoxylated tetraethylene- pentaimine	3.00
SRP	0.30

Protease ¹	0.069
Amylase ³	0.06
Cellulase	0.08
Lipase	0.18
Brightener	0.10
Minors/inerts to 100%	

Example 35
Liquid Fabric Cleaning Composition

The following liquid fabric cleaning composition of particular utility under Japanese machine wash conditions and for fine fabrics is prepared in accordance with the invention:

Component	35
AE2.5S	2.16
AS	3.30
N-Cocoyl N-methyl glucamine	1.10
Nonionic surfactant	10.00
Citric acid	0.40
Fatty acid	0.70
Base	0.85
Monoethanolamine	1.01
1,2-Propanediol	1.92
EtOH	0.24
HXS	2.09

Protease ¹	0.01
Amylase ³	0.06
Minors/inerts to 100%	

#### **Bar Fabric Cleaning Compositions**

Bar fabric cleaning compositions of the present invention suitable for handwashing soiled fabrics typically contain an effective amount of one or more protease enzymes, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight active protease enzyme of the composition. (See U.S. Patent No. 5,679,630 Examples).

Example 36
Bar Fabric Cleaning Compositions

Example No. Component C D Protease¹ 0.3 0.1 0.02 Protease² 0.4 0.1 Amylase³ 0.01 0.02 0.002 0.005 C₁₂-C₁₆ alkyl sulfate, Na 20.0 20.0 20.0 20.00 C₁₂-C₁₄ N-methyl glucamide 5.0 5.0 5.0 5.00 C₁₁-C₁₃ alkyl benzene sulfonate, Na 10.0 10.0 10.0 10.00 Sodium carbonate 25.0 25.0 25.0 25.00 Sodium tripolyphosphate 7.0 7.0 7.0 7.00 Zeolite A (0.1-.10µ) 5.0 5.0 5.0 5.00 Carboxymethylcellulose 0.2 0.2 0.2 0.20 Polyacrylate (MW 1400) 0.2 0.2 0.2 0.20 Coconut monethanolamide 5.0 5.0 5.0 5.00 Brightener, perfume 0.2 0.2 0.2 0.20 CaSO₄ 1.0 1.0 1.0 1.00 MgSO₄ 1.0 1.0 1.0 1.00 Water 4.0 4.0 4.0 4.00 Filler* balance to 100%

^{*}Can be selected from convenient materials such as CaCO3, talc, clay, silicates, and the like.

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

In Examples 36 C and D any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of the invention.

The compositions of the present invention can be suitably prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. 5,691,297 Nassano et al., issued November 11, 1997; U.S. 5,574,005 Welch et al., issued November 12, 1996; U.S. 5,569,645 Dinniwell et al., issued October 29, 1996; U.S. 5,565,422 Del Greco et al., issued October 15, 1996; U.S. 5,516,448 Capeci et al., issued May 14, 1996; U.S. 5,489,392 Capeci et al., issued February 6, 1996; U.S. 5,486,303 Capeci et al., issued January 23, 1996 all of which are incorporated herein by reference.

In addition to the above examples, the cleaning compositions of the present invention can be formulated into any suitable laundry detergent composition, non-limiting examples of which are described in U.S. 5,679,630 Baeck et al., issued October 21, 1997; U.S. 5,565,145 Watson et al., issued October 15, 1996; U.S. 5,478,489 Fredj et al., issued December 26, 1995; U.S. 5,470,507 Fredj et al., issued November 28, 1995; U.S. 5,466,802 Panandiker et al., issued November 14, 1995; U.S. 5,460,752 Fredj et al., issued October 24, 1995; U.S. 5,458,810 Fredj et al., issued October 17, 1995; U.S. 5,458,810 Fredj et al., issued February 22, 1994 all of which are incorporated herein by reference.

Having described the invention in detail with reference to preferred embodiments and the examples, it will be clear to those skilled in the art that various changes and modifications may be made without departing from the scope of the invention and the invention is not to be considered limited to what is described in the specification.

#### WHAT IS CLAIMED IS:

- 1. A fabric and/or dishwashing and/or hard surface cleaning composition comprising:
- (a) an effective amount of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin;
- (b) an amylase variant wherein said amylase variant is selected from the group consisting of:
- (i)  $\alpha$ -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas[®]  $\alpha$ -amylase activity assay and/or;
- (ii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv) α-amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-

Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;

- (v)  $\alpha$ -amylase according to (i-iv) wherein the  $\alpha$ -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi)  $\alpha$ -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii)  $\alpha$ -amylase showing positive immunological cross-reactivity with antibodies raised against an  $\alpha$ -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent  $\alpha$ -amylase, wherein the parent  $\alpha$ -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an  $\alpha$ -amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an  $\alpha$ amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent \(\alpha\)-amylase has been deleted; and/or (B) at least one amino acid residue of said parent α-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent  $\alpha$ -amylase; said variant having an  $\alpha$ -amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or  $\alpha$ -amylolytic activity at neutral to relatively high pH values; increased  $\alpha$ -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for  $\alpha$ -amylase variant to the pH of the medium; and
  - (c) one or more cleaning adjunct materials.
- 2. The cleaning composition according to Claim 1 wherein said protease variant is derived from a *Bacillus* subtilisin, preferably *Bacillus lentus* subtilisin or subtilisin 309.
- 3. The cleaning composition according to Claim 1 wherein said protease variant includes substitutions of the amino acid residues at position 103 and at one or more of the following positions 236 and 245, preferably at positions 103 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 248, 252, 257, 260, 270 and 275 or at positions 103 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 170, 183, 185, 205, 209, 210, 211, 212, 213, 215,

217, 222, 230, 232, 248, 252, 257, 260, 261, 270 and 275, more preferably at positions 103, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 248, 252, 257, 260, 270 and 275.

The cleaning composition according to Claim 1 wherein said protease variant includes a substitution set selected from the group consisting of:

12/102/103/104/159/212/232/236/245/248/252; 12/76/103/104/130/170/185/222/243/245;

12/76/103/104/130/222/245/261;

12/76/103/104/222/245;

12/76/103/104/130/222/245;

61/68/103/104/159/232/236/245/248/252;

62/103/104/159/213/232/236/245/248/252;

62/103/104/109/159/213/232/236/245/248/252; 62/103/104/159/232/236/245/248/252;

62/101/103/104/159/212/213/232/236/245/248/252;

62/103/104/130/159/213/232/236/245/248/252;

68/103/104/159/232/236/245/248/252/270;

68/103/104/159/185/232/236/245/248/252; 68/103/104/159/210/232/236/245/248/252;

68/103/104/159/185/210/232/236/245/248/252; 68/103/104/159/213/232/236/245/248/252;

68/103/104/159/230/232/236/245;

68/76/103/104/159/209/232/236/245:

68/103/104/232/236/245/248/257/275;

68/103/104/213/232/236/245/248/252;

68/103/104/159/232/236/245/248/252;

68/103/104/159/209/232/236/245;

68/76/103/104/159/236;

68/76/103/104/159/236/245;

68/76/103/104/159/232/236/245;

68/103/104/159/232/236/245/252;

68/103/104/159/232/236/245;

68/103/104/159/232/236/245/257;

68/76/103/104/159/211/232/236/245;

68/76/103/104/159/215/232/236/245;

68/103/104/159/210/232/236/245;

68/103/104/159/213/232/236/245/260;

68/76/103/104/159/213/232/236/245/260;

68/103/104/159/236;

68/76/103/104/159/210/232/236/245/260;

68/103/104/159/236/245;

68/103/104/159/183/232/236/245/248/252;

68/76/103/104/159/236/245;

68/103/104/232/236/245/257/275;

68/103/104/159/213/232/236/245;

76/103/222/245;

76/103/104/159/232/236/245;

76/103/104/159/213/232/236/245/260;

76/103/104/159;

76/103/104/131/159/232/236/245/248/252;

76/103/104/222/245;

97/103/104/159/232/236/245/248/252;

98/102/103/104/159/212/232/236/245/248/252; 98/103/104/159/232/236/245/248/252;

101/103/104/159/232/236/245/248/252:

102/103/104/159/232/236/245/248/252;

103/104/159/232/236/245;

103/104/159/232/236/245/248/252;

103/104/159/205/209/232/236/245/257

103/104/159/232/245/248/252;

 103/104/159/205/209/210/232/236/245/257;
 103/104/159/213/232/236/245/248/252;

 103/104/159/217/232/236/245/248/252;
 103/104/130/159/232/236/245/248/252;

 103/104/159/230/236/245;
 103/104/159/236/245;

 103/104/159/248/252/270;
 103/104/131/159/232/236/245/248/252;

103/104/159/205/209/232/236/245; and

103/104/159/232/236/245/257.

5. The cleaning composition according to Claim 4 wherein said protease variant includes a substitution set selected from the group consisting of:

12R/76D/103A/104T/130T/222S/245R; 12R/76D/103A/104I/222S/245R;

12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;

12R/76D/103A/104T/130G/222S/245R/261D;

12R/76D/103A/104T/130G/170S/185D/222S/243D/245R;

61E/68A/103A/104I/159D/232V/236H/245R/248D/252K;

62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K;

62D/103A/104I/159D/213R/232V/236H/245R/248D/252K;

62D/103A/104I/159D/232V/236H/245R/248D/252K;

62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K;

62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K;

68A/76D/103A/104I/159D/213R/232V/236H/245R/260A;

68A/103A/104I/159D/236H:

68A/103A/104I/159D/236H/245R;

68A/76D/103A/104I/159D/210I/232V/236H/245R/260A;

68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/209W/232V/236H/245R:

68A/76D/103A/104I/159D/211R/232V/236H/245R;

68A/76D/103A/104I/159D/215R/232V/236H/245R;

68A/103A/104I/159D/213R/232V/236H/245R/260A;

68A/76D/103A/104I/159D/236H;

68A/76D/103A/104I/159D/236H/245R:

68A/76D/103A/104I/159D/232V/236H/245R;

68A/103A/104I/159D/232V/236H/245R/252K:

68A/103A/104I/159D/232V/236H/245R:

68A/103A/104I/159D/232V/236H/245R/257V;

68A/103A/104I/159D/185D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/210L/232V/236H/245R/248D/252K;

68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K;

68A/103A/104I/159D/213E/232V/236H/245R/248D/252K: 68A/103A/104I/159D/230V/232V/236H/245R; 68A/76D/103A/104I/159D/209W/232V/236H/245R: 68A/103A/104I/232V/236H/245R/248D/257V/275H; 68A/103A/104I/232V/236H/245R/257V/275H: 68A/103A/104I/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210I/232V/236H/245R; 68A/103A/104I/159D/210L/232V/236H/245R: 68A/103A/104I/159D/213G/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/248D/252K/270A; 76D/103A/222S/245R: 76D/103A/104I/159D/232V/236H/245R: 76D/103A/104I/159D: 76D/103A/104I/222S/245R; 76D/103A/104I/131V/159D/232V/236H/245R/248D/252K; 76D/103A/104I/159D/213R/232V/236H/245R/260A; 97E/103A/104I/159D/232V/236H/245R/248D/252K; 98L/103A/104I/159D/232V/236H/245R/248D/252K; 98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 101G/103A/104I/159D/232V/236H/245R/248D/252K: 102A/103A/104I/159D/232V/236H/245R/248D/252K: 103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/213R/232V/236H/245R/248D/252K; 103A/104I/130G/159D/232V/236H/245R/248D/252K; 103A/104I/159D/230V/236H/245R: 103A/104I/159D/217E/232V/236H/245R/248D/252K; 103A/104I/159D/236H/245R; 103A/104I/159D/248D/252K/270V: 103A/104I/159D/232V/236H/245R; 103A/104I/159D/205I/209W/232V/236H/245R; 103A/104I/159D/232V/236H/245R/257V: 103A/104I/159D/205I/209W/232V/236H/245R/257V; 103A/104I/131V/159D/232V/236H/245R/248D/252K; 103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and

103A/104I/159D/232V/245R/248D/252K.

- 6. The cleaning composition according to Claim 1 wherein said cleaning adjunct materials are selected from the group consisting of surfactants, solvents, buffers, enzymes, soil release agents, clay soil removal agents, dispersing agents, brighteners, suds suppressors, fabric softeners, suds boosters, enzyme stabilizers, builders, other bleaching agents, dyes, perfumes, chelants and mixtures thereof.
- 7. The cleaning composition according to Claim 6 wherein said cleaning adjunct materials comprise at least one detersive surfactant, preferably a branched surfactant, more preferably a mid-chained branched surfactant.
- 8. The cleaning composition according to Claim 7 wherein the cleaning adjunct materials comprise at least about 0.1% surfactant by weight of the composition, said surfactant comprising materials selected from the group consisting of alkyl benzene sulfonates, primary alkyl sulfates, secondary alkyl sulfates, alkyl alkoxy sulfates, alkyl alkoxy carboxylates, alkyl polyglycosides and their corresponding sulfated polyglycosides, alpha-sulfonated fatty acid esters, alkyl and alkyl phenol alkoxylates, betaines and sulfobetaines, amine oxides, N-methyl glucamides, nonionic primary alcohol ethoxylates, nonionic primary alcohol mixed ethoxy/propoxy, and mixtures thereof.
- 9. The cleaning composition according to Claim 8 further comprising at least about 5% builder selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof.
- 10. The cleaning composition according to Claim 6 wherein said cleaning adjunct materials comprise at least one detersive enzyme selected from the group consisting of cellulases, lipases, other amylases, phospholipases, other proteases, peroxidases and mixtures thereof.
- 11. The cleaning composition according to Claim 6 wherein said cleaning adjunct materials comprise at least one bleaching agent preferably selected from the group consisting of percarbonates, perborates and mixtures thereof, and optionally further comprising at least one bleach activator preferably selected from the group consisting of benzoyloxybenzenesulphonate (BOBS), nonanoyloxybenzenesulphonate (NOBS), decanoyloxybenzenesulphonate (C₁₀-OBS), octanoyloxybenzenesulphonate (C₈-OBS), perhydrolyzable esters, 4-[N-(nonaoyl) amino hexanoyloxy]-benzene sulfonate sodium salt (NACA-OBS), lauryloxybenzenesulphonate (LOBS or C₁₂-OBS), 10-undecenoyloxybenzenesulfonate (UDOBS or C₁₁-OBS with unsaturation in the 10

position), and decanoyloxybenzoic acid (DOBA) and mixtures thereof, and further optionally comprising a bleach catalyst, preferably 3-(3,4-dihydroisoquinolinium) propane sulfonate.

- 12. The cleaning composition according to Claim 1 wherein said cleaning composition is a fabric cleaning composition, preferably in the form of a liquid, granule, bar, tablet, gel, powder or foam, comprising at least about 5% surfactant and at least about 5% builder by weight of the composition.
- 13. The cleaning composition according to Claim 1 wherein said cleaning composition is a fabric cleaning composition comprising:
  - (a) from about 0.0001% to about 10% by weight of said protease variant;
  - (b) from about 0.0001% to about 0.1% by weight of said amylase variant;
- (c) at least about 5% by weight of a surfactant preferably selected from the group consisting of alkyl benzene sulfonates, primary alkyl sulfates, secondary alkyl sulfates, alkyl alkoxy sulfates, alkyl alkoxy carboxylates, alkyl polyglycosides and their corresponding sulfated polyglycosides, alpha-sulfonated farry acid esters, alkyl and alkyl phenol alkoxylates, betaines and sulfobetaines, amine oxides, N-methyl glucamides, nonionic primary alcohol ethoxylates, nonionic primary alcohol mixed ethoxy/propoxy, and mixtures thereof; and wherein further the builder is selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof; and
- (d) at least about 5% by weight of a builder preferably selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof.
- 14. The cleaning composition according to Claim 25 is in the form of a concentrated granular fabric cleaning composition comprising at least about 15% surfactant.
- 15. A method for cleaning fabric, said method comprising contacting a fabric in need of cleaning with a cleaning composition according to Claims 12 or 13.
- 16. The cleaning composition according to Claim 1 wherein said cleaning composition is a dishwashing composition, preferably in the form of a liquid, granule, powder, gel or tablet, comprising:
  - (a) from about 0.0001% to about 10% by weight of said protease variant;
- (b) from about 0.0001% to about 0.1% by weight of the dishwashing composition of said amylase variant; and
  - (c) from about 0.1% to about 10% by weight of a surfactant.

17. A method for cleaning dishes, said method comprising contacting a dish in need of cleaning with a cleaning composition according to Claim 16.

### 18. A personal cleansing composition comprising:

- (a) an effective amount of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin;
- (b) an amylase variant wherein said amylase variant is selected from the group consisting of:
- (i)  $\alpha$ -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas[®]  $\alpha$ -amylase activity assay and/or;
- (ii) α-amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an α-amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv) α-amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-

Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;

- (v)  $\alpha$ -amylase according to (i-iv) wherein the  $\alpha$ -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi)  $\alpha$ -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii)  $\alpha$ -amylase showing positive immunological cross-reactivity with antibodies raised against an  $\alpha$ -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent  $\alpha$ -amylase, wherein the parent  $\alpha$ -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an  $\alpha$ -amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an  $\alpha$ amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent \(\alpha\)-amylase has been deleted; and/or (B) at least one amino acid residue of said parent α-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent  $\alpha$ -amylase; said variant having an  $\alpha$ -amylase activity and exhibiting at least one of the following properties relative to said parent  $\alpha$ -amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or  $\alpha$ -amylolytic activity at neutral to relatively high pH values; increased  $\alpha$ -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pl) so as to better match the pl value for \alpha-amylase variant to the pH of the medium; and
  - (c) one or more cleaning adjunct materials.
- 19. The personal cleansing composition according to Claim 18 wherein said personal cleansing composition comprises:
- (a) from about 0.001% to about 5%, preferably from about 0.001% to about 2%, more preferably from about 0.002% to about 0.8% by weight of said protease variant;
- (b) from about 0.0001% to about 0.1% by weight of the personal cleansing composition of said amylase variant; and
- (c) from about 0.1% to about 95% by weight of a surfactant system preferably comprising a surfactant selected from the group consisting of anionic carboxylates, amine oxides, alkyl glucosides, glucose amides, alkyl sulfates, alkyl ether sulfates, acyl isethionates, alkyl sulfosuccinates, alkyl phosphate esters, ethoxylated phosphate esters,

alkyl glyceryl ether sulfonates and mixtures thereof, more preferably comprising a surfactant selected from the group consisting of soaps, acylglutamates, alkyl sarcosinates, lauramine oxides, cocamine oxides, cocamindopropylamine oxides, decylglucosides, lauryl sulfates, laureth sulfates, C₁₂₋₁₈ acyl isethionates and mixtures thereof; and

- (d) optionally, from about 0.05% to about 50% by weight of an enzyme stabilizer.
- 20. The personal cleansing composition according to Claim 19 wherein said surfactant is soap at a level of at least about 2%, preferably at least about 10%, more preferably at least about 25% by weight of the cleaning composition.
- 21. The personal cleansing composition according to Claim 20 wherein the ratio of soap to protease variant is from about 2,000:1 to about 8:1, preferably from about 400:1 to about 40:1.
- 22. A method for personal cleansing, said method comprising contacting a part of the human or lower animal body in need of cleaning with a cleaning composition according to Claim 18.
- 23. A fabric and/or dishwashing and/or hard surface cleaning composition comprising:
- (a) an effective amount of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;
- (b) an amylase variant wherein said amylase variant is selected from the group consisting of:
- (i)  $\alpha$ -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas®  $\alpha$ -amylase activity assay and/or;
- (ii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;

- (iv)  $\alpha$ -amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;
- (v)  $\alpha$ -amylase according to (i-iv) wherein the  $\alpha$ -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi)  $\alpha$ -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii)  $\alpha$ -amylase showing positive immunological cross-reactivity with antibodies raised against an  $\alpha$ -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent  $\alpha$ -amylase, wherein the parent  $\alpha$ -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an  $\alpha$ -amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an  $\alpha$ amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent α-amylase has been deleted; and/or (B) at least one amino acid residue of said parent α-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α-amylase; said variant having an α-amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or  $\alpha$ -amylolytic activity at neutral to relatively high pH values; increased  $\alpha$ -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for α-amylase variant to the pH of the medium; and
  - (c) one or more cleaning adjunct materials.
- 24. The cleaning composition according to Claim 23 wherein said protease variant is derived from a *Bacillus* subtilisin, preferably *Bacillus* lentus subtilisin or subtilisin 309.
- 25. The cleaning composition according to Claim 23 wherein said protease variant includes substitutions of the amino acid residues at one or more of the following positions selected from the group consisting of:
- 1) position 62 and at one or more of the following positions 103, 104, 109, 159, 213, 232, 236, 245, 248 and 252;

- 2) position 212 and at one or more of the following positions 12, 98, 102, 103, 104, 159, 232, 236, 245, 248 and 252;
- 3) position 230 and at one or more of the following positions 68, 103, 104, 159, 232, 236 and 245;
- 4) position 232 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 5) position 232 and at one or more of the following positions 103, 104, 236 and 245;
- 6) positions 232 and 103 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 7) positions 232 and 104 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 8) positions 232 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 9) positions 232 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 10) positions 232, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 11) position 252 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 12) position 252 and at one or more of the following positions 103, 104, 236 and 245;
- 13) positions 252 and 103 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 14) positions 252 and 104 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;

- 15) positions 252 and 236 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 16) positions 252 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 17) positions 252, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 18) position 257 and at one or more of the following positions 68, 103, 104, 205, 209, 210, 232, 236, 245 and 275.
- 26. The cleaning composition according to Claim 23 wherein said protease variant includes a substitution set selected from the group consisting of: 12/102/103/104/159/212/232/236/245/248/252; 61/68/103/104/159/213/232/236/245/248/252; 62/103/104/130/159/213/232/236/245/248/252; 62/103/104/159/213/232/236/245/248/252;

 $62/103/104/109/159/213/232/236/245/248/252; \ 62/103/104/159/232/236/245/248/252; \\$ 

62/101/103/104/159/212/213/232/236/245/248/252;

68/103/104/159/232/236/245/248/252/270;

68/103/104/159/185/232/236/245/248/252; 68/103/104/159/210/232/236/245/248/252;

68/103/104/159/185/210/232/236/245/248/252; 68/103/104/159/213/232/236/245/248/252;

68/103/104/159/230/232/236/245; 68/76/103/104/159/209/232/236/245;

68/103/104/232/236/245/248/257/275; 68/103/104/213/232/236/245/248/252;

68/103/104/159/232/236/245/248/252; 68/103/104/159/209/232/236/245; 68/76/103/104/159/232/236/245; 68/76/103/104/159/232/236/245;

68/76/103/104/159/232/236/245; 68/103/104/159/232/236/245/252; 68/103/104/159/232/236/245/252;

68/103/104/159/232/236/245; 68/103/104/159/232/236/245/257; 68/76/103/104/159/211/232/236/245; 68/76/103/104/159/215/232/236/245

68/76/103/104/159/211/232/236/245; 68/76/103/104/159/215/232/236/245; 68/103/104/159/210/232/236/245; 68/103/104/159/213/232/236/245/260;

68/76/103/104/159/213/232/236/245/260; 68/76/103/104/159/210/232/236/245/260;

68/103/104/159/183/232/236/245/248/252; 68/103/104/232/236/245/257/275;

68/103/104/159/213/232/236/245; 76/103/104/159/232/236/245;

76/103/104/159/213/232/236/245/260; 76/103/104/131/159/232/236/245/248/252;

97/103/104/159/232/236/245/248/252; 98/103/104/159/232/236/245/248/252;

98/102/103/104/159/212/232/236/245/248/252; 101/103/104/159/232/236/245/248/252;

102/103/104/159/232/236/245/248/252; 103/104/159/232/236/245;

103/104/159/248/252/270; 103/104/159/232/236/245/248/252;

103/104/159/205/209/232/236/245/257 103/104/159/232/245/248/252;

103/104/159/205/209/210/232/236/245/257; 103/104/159/213/232/236/245/248/252; 103/104/131/159/232/236/245/248/252; 103/104/131/159/232/236/245/248/252; 103/104/131/159/232/236/245/248/252; 103/104/159/205/209/232/236/245; and

103/104/159/232/236/245/257.

27. The cleaning composition according to Claim 26 wherein said protease variant includes a substitution set selected from the group consisting of:

12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 61E/68A/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K; 62D/103A/104I/159D/213R/232V/236H/245R/248D/252K; 62D/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K; 62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K; 68A/76D/103A/104I/159D/213R/232V/236H/245R/260A; 68A/76D/103A/104I/159D/210I/232V/236H/245R/260A; 68A/103A/104I/159D/183D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/209W/232V/236H/245R; 68A/76D/103A/104I/159D/211R/232V/236H/245R; 68A/76D/103A/104I/159D/215R/232V/236H/245R; 68A/103A/104I/159D/213R/232V/236H/245R/260A; 68A/76D/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/252K; 68A/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/257V; 68A/103A/104I/159D/185D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/230V/232V/236H/245R; 68A/76D/103A/104I/159D/209W/232V/236H/245R; 68A/103A/104I/232V/236H/245R/248D/257V/275H; 68A/103A/104I/232V/236H/245R/257V/275H; 68A/103A/104I/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210I/232V/236H/245R; 68A/103A/104I/159D/210L/232V/236H/245R;

68A/103A/104I/159D/213G/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/248D/252K/270A; 76D/103A/104I/159D/232V/236H/245R; 76D/103A/104I/131V/159D/232V/236H/245R/248D/252K; 76D/103A/104I/159D/213R/232V/236H/245R/260A; 97E/103A/104I/159D/232V/236H/245R/248D/252K; 98L/103A/104I/159D/232V/236H/245R/248D/252K; 98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 101G/103A/104I/159D/232V/236H/245R/248D/252K; 102A/103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/213R/232V/236H/245R/248D/252K; 103A/104I/130G/159D/232V/236H/245R/248D/252K; 103A/104I/159D/217E/232V/236H/245R/248D/252K; 103A/104I/159D/248D/252K/270V; 103A/104I/159D/232V/236H/245R; 103A/104I/159D/205I/209W/232V/236H/245R; 103A/104I/159D/232V/236H/245R/257V; 103A/104I/159D/205I/209W/232V/236H/245R/257V; 103A/104I/131V/159D/232V/236H/245R/248D/252K; 103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and 103A/104I/159D/232V/245R/248D/252K.

- 28. The cleaning composition according to Claim 23 wherein said cleaning adjunct materials are selected from the group consisting of surfactants, solvents, buffers, enzymes, soil release agents, clay soil removal agents, dispersing agents, brighteners, suds suppressors, fabric softeners, suds boosters, enzyme stabilizers, builders, other bleaching agents, dyes, perfumes, chelants and mixtures thereof.
- 29. The cleaning composition according to Claim 28 wherein said cleaning adjunct materials comprise at least one detersive surfactant, preferably a branched surfactant, more preferably a mid-chained branched surfactant.
- 30. The cleaning composition according to Claim 28 wherein the cleaning adjunct materials comprise at least about 0.1% surfactant by weight of the composition, said surfactant comprising materials selected from the group consisting of alkyl benzene sulfonates, primary alkyl sulfates, secondary alkyl sulfates, alkyl alkoxy sulfates, alkyl

alkoxy carboxylates, alkyl polyglycosides and their corresponding sulfated polyglycosides, alpha-sulfonated fatty acid esters, alkyl and alkyl phenol alkoxylates, betaines and sulfobetaines, amine oxides, N-methyl glucamides, nonionic primary alcohol ethoxylates, nonionic primary alcohol mixed ethoxy/propoxy, and mixtures thereof.

- 31. The cleaning composition according to Claim 30 further comprising at least about 5% builder selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof.
- 32. The cleaning composition according to Claim 28 wherein said cleaning adjunct materials comprise at least one detersive enzyme selected from the group consisting of cellulases, lipases, amylases, phospholipases, other proteases, peroxidases and mixtures thereof.
- 33. The cleaning composition according to Claim 28 wherein said cleaning adjunct materials comprise at least one bleaching agent preferably selected from the group consisting of percarbonates, perborates and mixtures thereof, and optionally further comprising at least one bleach activator preferably selected from the group consisting of benzoyloxybenzenesulphonate (BOBS), nonanoyloxybenzenesulphonate (NOBS), decanoyloxybenzenesulphonate (C₈-OBS), octanoyloxybenzenesulphonate (C₈-OBS), perhydrolyzable esters, 4-[N-(nonaoyl) amino hexanoyloxy]-benzene sulfonate sodium salt (NACA-OBS), lauryloxybenzenesulphonate (LOBS or C₁₂-OBS), 10-undecenoyloxybenzenesulfonate (UDOBS or C₁₁-OBS with unsaturation in the 10 position), and decanoyloxybenzoic acid (DOBA) and mixtures thereof, and further optionally comprising a bleach catalyst, preferably 3-(3,4-dihydroisoquinolinium) propane sulfonate.
- 34. The cleaning composition according to Claim 23 wherein said cleaning composition is a fabric cleaning composition, preferably in the form of a liquid, granule, bar, tablet, gel, powder or foam, comprising at least about 5% surfactant and at least about 5% builder by weight of the composition.
- 35. The cleaning composition according to Claim 23 wherein said cleaning composition is a fabric cleaning composition comprising:
  - (a) from about 0.0001% to about 10% by weight of said protease variant;
- (b) from about 0.0001% to about 0.1% by weight of the fabric cleaning composition of said amylase variant;

- (c) at least about 5% by weight of a surfactant preferably selected from the group consisting of alkyl benzene sulfonates, primary alkyl sulfates, secondary alkyl sulfates, alkyl alkoxy sulfates, alkyl alkoxy carboxylates, alkyl polyglycosides and their corresponding sulfated polyglycosides, alpha-sulfonated farry acid esters, alkyl and alkyl phenol alkoxylates, betaines and sulfobetaines, amine oxides, N-methyl glucamides, nonionic primary alcohol ethoxylates, nonionic primary alcohol mixed ethoxy/propoxy, and mixtures thereof; and wherein further the builder is selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof; and
- (d) at least about 5% by weight of a builder preferably selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof.
- 36. The cleaning composition according to Claim 35 is in the form of a concentrated granular fabric cleaning composition comprising at least about 15% surfactant.
- 37. A method for cleaning fabric, said method comprising contacting a fabric in need of cleaning with a cleaning composition according to Claims 34 or 35.
- 38. The cleaning composition according to Claim 23 wherein said cleaning composition is a dishwashing composition, preferably in the form of a liquid, granule, powder, gel or tablet, comprising:
  - (a) from about 0.0001% to about 10% by weight of said protease variant; and
  - (b) from about 0.1% to about 10% by weight of a surfactant.
- 39. A method for cleaning dishes, said method comprising contacting a dish in need of cleaning with a cleaning composition according to Claim 38.
- 40. A personal cleansing composition comprising:
- (a) an effective amount of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;
- (b) an amylase variant wherein said amylase variant is selected from the group consisting of:
- (i)  $\alpha$ -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas[®]  $\alpha$ -amylase activity assay and/or;

- (ii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii)  $\alpha$ -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv)  $\alpha$ -amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an  $\alpha$ -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;
- (v)  $\alpha$ -amylase according to (i-iv) wherein the  $\alpha$ -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi) α-amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii)  $\alpha$ -amylase showing positive immunological cross-reactivity with antibodies raised against an  $\alpha$ -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent  $\alpha$ -amylase, wherein the parent  $\alpha$ -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an α-amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an  $\alpha$ amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent \alpha-amylase has been deleted; and/or (B) at least one amino acid residue of said parent \(\alpha\)-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α-amylase; said variant having an α-amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or α-amylolytic activity at neutral to relatively high pH values; increased α-amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pl) so as to better match the pI value for α-amylase variant to the pH of the medium; and
  - (c) one or more cleaning adjunct materials.
- 41. The personal cleansing composition according to Claim 40 wherein said personal cleansing composition comprises:

- (a) from about 0.001% to about 5%, preferably from about 0.001% to about 2%, more preferably from about 0.002% to about 0.8% by weight of said protease variant;
- (b) from about 0.0001% to about 0.1% by weight of the personal cleansing composition of said amylase variant; and
- (c) from about 0.1% to about 95% by weight of a surfactant system preferably comprising a surfactant selected from the group consisting of anionic carboxylates, amine oxides, alkyl glucosides, glucose amides, alkyl sulfates, alkyl ether sulfates, acyl isethionates, alkyl sulfosuccinates, alkyl phosphate esters, ethoxylated phosphate esters, alkyl glyceryl ether sulfonates and mixtures thereof, more preferably comprising a surfactant selected from the group consisting of soaps, acylglutamates, alkyl sarcosinates, lauramine oxides, cocamine oxides, cocamindopropylamine oxides, decylglucosides, lauryl sulfates, laureth sulfates,  $C_{12-18}$  acyl isethionates and mixtures thereof; and
- (d) optionally, from about 0.05% to about 50% by weight of an enzyme stabilizer.
- 42. The personal cleansing composition according to Claim 41 wherein said surfactant is soap at a level of at least about 2%, preferably at least about 10%, more preferably at least about 25% by weight of the cleaning composition.
- The personal cleansing composition according to Claim 42 wherein the ratio of soap to protease variant is from about 2,000:1 to about 8:1, preferably from about 400:1 to about 40:1.
- 44. A method for personal cleansing, said method comprising contacting a part of the human or lower animal body in need of cleaning with a cleaning composition according to Claim 40.
- 45. A method for pretreating a fabric in need of cleaning, said method comprising contacting said fabric prior to washing said fabric with an aqueous solution containing a surfactant with a bleaching composition according to Claims 12 or 13.
- 46. A method for pretreating a fabric in need of cleaning, said method comprising contacting said fabric prior to washing said fabric with an aqueous solution containing a surfactant with a bleaching composition according to Claims 34 or 35.

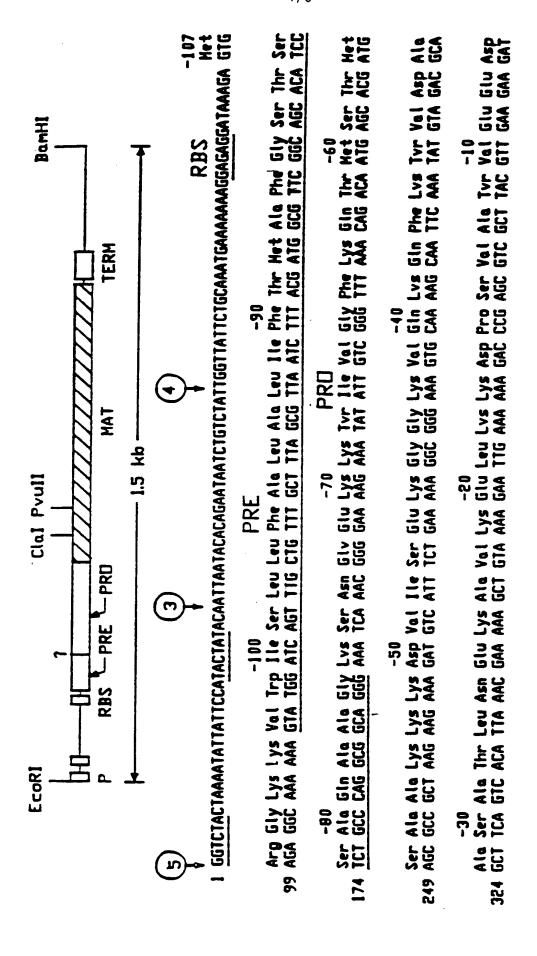


FIG.-1A

# F1G-11

GC V Ala Val GCT GTA 1vs AAG Ser TCT Ala Asn GCA AAC The HIS Ala 1×1 152 142 EAC 90' Leu Tvr CTT TAC AAA LEu C16 Asn GAT 61v 66A 140 Asp GAT Va l G1G A C Ala GCT 2 £ 13 Ser HIS TOT TOT CAC Ala A Ser His Leu Lvs Ala Ala Val TTA AAA GCG GCA GTT AG T Pr 173 Ser AGC Ala I 다. 다음 Ser GCA GCA Ser TCT Asp Asn Asn AAC AAC ILE LYS ( Glu Ser TCA GLY Val Ala Pro Ser GGC GTT GCG CCA AGC Ser TCT A1C Ser Asp GAT 10 Val Ser Gln 1 GTA TCA CAA A 160 61y 660 60 Asp GAC 11e ATC 657 657 Ile Ile Asn I Ala GCT 61y 661 Gln CAA Jer 100 Ala Phe 170 Ser 1hr ACT Ser AĞC Val Leu G 11e Asp ATC GAC Ser TCT 65 66 66 66 67 Asn Pro CCT 61y 660 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 July 1.00 J Pro Thr Asn ACA AAT Ala Ala Gly Asn Glu GCA GCC GGT AAC GAA 61y 661 -1| 1 His Ala Tyr Ala Gln Ser Val Pro Tyr CAT GCG TAC GCG CAG TCC GTG CCT TAC Ser Ser 101 30 Val 611 86 667 661 Asn Ser 11e | AAC TCA ATC 1 Gln Tyr CAA TAC Pro CCT Gtu GAA Asn Val Lys Val Ala AAT GTT AAA GTA GCG Ser 101 61y 66A ٦ د ت 99 Met Ser Leu ( ATG AGC CTC ( 50 Het Val | ATG GTT ( Ala GCG Ser 100 Asn AAT **1**50 150 Val 100 61y 661 Ala Ata Asp GAC Val GTC Ser Tra Ser AGC ASI Ala Asp Ata GCT Val GIA ה ה ה ה Ala Va l GTT A le ۲ها 570 **≥8**6 5 5 5 5 5 AT ACT Val Leu GTT CTC Val GT1 65 660 Bo L GTA SEY SEY ACA ACA があ 120 Asp GAC Ser TCC 2 65 65 65 2 2 2 2 2 2 2 3 849 549 624 774 399 474

AACATAAAAAACCGGCCTTGGCCCCGCGGTTTTTTATTATTTTTCTTCCTCCGCATGTTCAATCCGCTCC 220 Thr Ser Met Ala Ser Pro His Val Ala Gly Aal Ala Leu Ile Leu Ser Lvs His Pro Asn Irp Thr Asn Thr ACG TCA ATG GCA TCT CCG CAC GTT GCC GGA GCG GCT GCT TTG ATT CTT TCT AAG CAC CCG AAC 1GG ASD AND AND Pro Ser Val Ile Ala Val Glv Ala Val Asp Ser Ser Asm Glm Arp Ala Ser Phe Ser Ser Vsl Glv CCT TCT GTC ATT GCA GTA GGC GCT GTT GAC AGC AGC CAA AGA GCA TCT TTC TCA AGC GTA GGA 200 Ala Pro Gly Val Ser Ile Gin Ser Thr Leu Pro Giv Asn Lvs Tvr Gly Ala Tvr Asn GCA CCT GGC GTA TCT ATC CAA AGC ACG CTT CCT GGA AAC AAA TAC GGG GCG TAC AAC ESO GIN GIN Val Aro Ser Ser Leu Glu Asn Thr Thr Thr Lvs Leu Gly Asp Ser Phe Tyr Tyr Glv Lvs Glv Leu Ile 1149 CAA GTC CGC AGC AGT TTA GAA AAC ACC ACT ACA AAA CTT GGT GAT TCT TTG TAC TAT GGA AAA GGG CTG ATC TERM 270 Val Gin Ala Ala Ala Gin DC GTA CAA GCG GCA GCT: CAG TAA Leu Asp Val Het CTT GAT GTC ATG Lvs Tvr F AAA TAC ( GAG 924 999 1074 1224

1316 ATAATCGACGGATGGCTCCCTCTGAAAATTTTAACGAGAAACGGCGGGTTGACCCGGCTCAGTCCCGTAACGGCCAACTCCTGAAACGTCTCAATCGCCG 1416 CTTCCCGGTTTCCGGTCAGCTCAATGCCATAACGGTCGGCGGTTTTCCTGATACCGGGAGACGGCATTCGTAATCGGATC

FIG.-1C

# CONSERVED RESIDUES IN SUBTILISING FROM BACILLUS AMYLOLIQUEFACIENS

1	Q	s	v	P	•	G	•	•	10	•	•	λ	P	λ	•	H	•	•	20 G
21	T	G	s	•	v	K	v	λ	30 V	٠.	D		G	•	•	•	•	H	40 P
41 D	L	•	•	•	C	G	λ	s	<b>5</b> 0	v	P	•	•	•	•	•	•	Q	<b>60</b> D
61 •	N	•	H	G	T	H	v	A	70 G	T	•	λ	λ	L	N	N	S	I	<b>G</b>
81 V	L	G	v	A	P	s	A	•	90 L	Y	λ	v	ĸ	v	L	G	λ	•	100 G
10: S		•	•	S		L	•	•	110 G	•	E	W	A	•	N	•	•	•	120
12: V	_	N	•	s	L	G	•	P	130 S		s	•	•	•	•	•	λ	•	140
14:	1.	•	•	•	G	v	•	v	150 V	A	λ	•	G	N	•	G	•	•	160
16:		•	•	•	•	Y	P	•	170 •		•	•	•	•	A	v	G	λ	180
18: D	1	•	N	•	•	A	S	F	190 S		•	G	•	•	L	D	•		200 À
20: P	_	v	•	•	Q	s	T	•	210 P		•	•	Y	•	•	•	N	G	220 T
22 S		λ	•	P	н	v	λ	G	230 A		A	L	•	•	•	K	•	•	240
24 W	1.	•	•	Q	•	R	•	•	250 L	•	N	T	•	•	•	L	G	•	260
26 •		Y	G	•	G	L	•	N	270		λ	λ	•	•					

FIG. 2

Comparison of subtilisin sequences from:

B.amyloliquefaciens	B.subtilis	licheniformis	lentus
B.am)	B.sul	B.11c	B.ler

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**FIG 3/A** 

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His His As	n Gly Thr As	n Gly Thr	Met N	Aet Gin Ty	r Phe Glu	Trp Tyr
1	5		10		15	
Leu Pro As	in Asp Gly A	sn His Tr	p Asn .	Arg Leu A	rg Asp A	sp Ala Ala
	20		25		30	
Asn Leu Ly	s Ser Lys G	ly lie Thr	Ala Va	i Trp lie Pr	o Pro Al	a Trp
3		40	-	45		
	r Ser Gin As	n Asp Val	Gly T	yr Gly Ala	Tyr Asp	Leu Tyr
50		55		6 0		
	ly Glu Phe A		s Gly T	Thr Val Arg	Thr Lys	Tyr Giy
65	70			75		80
Thr Arg As	n Gin Leu G	in Ala Ala	Val Ti	nr Ser Leu	Lys Asn	Asn Gly
	85		90		95	
	Tyr Gly Asp			n His Lys (Gly Gly A	la Asp
	100	105			110	
	ille Val Asn		Slu Val	Asn Arg 5	Ser Asn A	Arg Asn
115		120		125		
	r Ser Gly Glu				or Lys Ph	e Asp
130		135		140		
	y Arg Gly As				Trp Arg	Trp Tyr
145	150			155		160
HIS PHE AS	p Gly Thr As	sp Irp As				
la Tualia	165	Th. 61 1		70	175	
	Phe Arg Gly		.ys Ala	Trp Asp 7		'al Asp
		185		4 - • •	190	
195	n Gly Asn T	200	r Leu N		a Asp Va	! Asp Met
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Gly Ala lie G	ilu Asn Tyr Leu	Asn Lys Thr S	er Trp Asn H	lis Ser Val
275	28	10	285	
Phe Asp Val	Pro Leu His Ty	r Asn Leu Tyr	Asn Ala Ser	Asn Ser Giy
290	29)5	300	
Gly Tyr Tyr	Asp Met Arg As	in lie Leu Asn	Gly Ser Val	Val Gin Lys
305	310	31	5	320
His Pro Thr	His Ala Val Thr	Phe Val Asp A	sn His Asp S	Ser Gin Pro
	325	_ 3	30	335
Giy Giu Ala	Leu Glu Ser Phe	Val Gin Gin T	rp Phe Lys F	Pro Leu Ala
	340	345	350)
Tyr Ala Leu	Val leu Thr Arg	Glu Gln Gly T	yr Pro Ser V	al Phe Tyr
355	36	30	365	
Gly Asp Tyr	Tyr Gly lie Pro	Thr His Gly Va	al Pro Ala Me	et Lys Ser
370	375	380	D	
Lys lie Asp	Pro leu Leu Gin	Ala Arg Gin Ti	hr Phe Ala T	yr Gly Thr
385	390	395	;	400
Gln His Asp	Tyr Phe Asp Hi	s His Asp lie li	ie Gly Trp Th	nr Arg Glu
	405	410	41	5
Gly Asn Ser	Ser His Pro Ass	n Ser Gly Leu	Ala Thr lie M	let Ser Asp
	420	425	430	
Gly Pro Gly	Giy Asn Lys Tr	Met Tyr Val	Gly Lys Asn	Lys Ala Giy
43	5 44	40	445	
Gln Val Trp	Arg Asp Ile Thr	Gly Asn Arg	Thr Gly Thr \	√al Thr lie
450	455	4	60	
Asn Ala Ası	Gly Trp Gly A	sn Phe Ser Val	I Asn Gly Gly	/ Ser Val Sei
465	470	47	5	480
Val Trp Val	Lys Gln			
	485			

HIS HIS ASI	GIY INT AST	Gly Thr Me	t Met Gin	Tyr Phe	Glu Trp His
1	5		10		15
Leu Pro Asi	n Asp Gly As	n His Trp A	sn Arg Le	u Arg Ası	p Asp Ala Ser
	20		!5		30
Asn Leu Ar	g Asn Arg Gi	y lie Thr Ala	lie Trp II	e Pro Pro	Ala Trp
	35	40	_	45	•
Lys Gly Thr	Ser Gin Asn	Asp Val Gly	Tyr Gly	Ala Tyr A	SD Leu Tvr
50		5 5		60	.,
Asp Leu Gly	Glu Phe As	n Gin Lys Gi	y Thr Val	Arg Thr	Lvs Tvr Giv
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Thr Arg Ser	Gin Leu Glu	Ser Ala Ile I	His Ala Le	eu Lys Asi	n Asn Glv
80	85		90		95
Val Gin Val	Tyr Gly Asp	Val Val Met	Asn His	Lys Gly G	
	100	105		110	·
Ala Thr Glu	Asn Val Leu	Ala Val Glu	Val Asn	Pro Asn A	Asn Ara Asn
	115	120		125	i
Gin Glu lie S	Ser Gly Asp T	yr Thr lle G	iu Ala Trp	Thr Lys	Phe Asp
130		135	14	10	
Phe Pro Gly	Arg Gly Asn	Thr Tyr Ser	Asp Phe	Lys Trp	Arg Trp Tvr
145		150	15	55	
His Phe Asp	Gly Val Asp	Trp Asp Gli	n Ser Arg	Gin Phe	Gin Asn Arg
160	165		170		175
lle Tyr Lys P	he Arg Gly	Asp Gly Lys	Ala Tri As	sp Trp Glu	J Val Asp
	180	185		190	·
Ser Glu Asn	Giy Asn Tyr	Asp Tyr Lei	u Met Tyr	Ala Asp	Val Asp Met
	195	200		205	
Asp His Pro	Giu Val Val	Asn Glu Leu	Arg Arg	Trp Gly G	ilu Trp Tyr
210		215		220	
Thr Asn Thr	Leu Asn Leu	Asp Gly Ph	e Arg lie	Asp Ala \	/al Lys His
225		230	235		
lie Lys Tyr S	er Phe Thr A	rg Asp Trp	Leu Thr H	lis Val Arg	a Asn Ala
240	245		250		255
Thr Gly Lys	Glu Met Phe	Ala Val Ala	Glu Phe	Trp Lys A	sn Asp Leu
	260	265		27	
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Gly Ala Leu	Glu Asn Tyr Le	u Asn Lys Th	r Asn Trp Asn	His Ser Val	
	275	280	28		
Phe Asp Va	I Pro Leu His T	r Asn Leu Ty	r Asn Ala Ser	Asn Ser Glv	
29		295	300	,	
Gly Asn Ty	Asp Met Ala L	ys Leu Leu A:	sn Gly Thr Val	Val Gin Lvs	
305	31		315	,-	
His Pro Met	His Ala Val Th	Phe Val Asp	Asn His Asp	Ser Gin Pro	
320	325	38	30	335	
Gly Glu Ser	Leu Glu Ser Ph	e Val Gin Glu	Trp Phe Lys P	ro Leu Ala	
	340		345		350
Tyr Ala Leu	He Leu Thr Arg	Glu Gin Giy	Tyr Pro Ser Va	I Phe Tyr	
	355	360	365		
Gly Asp Tyr	Tyr Giy lie Pro	Thr His Ser \	/al Pro Ala Me	t Lys Ala	
370	;	375	380		
Lys lie Asp	Pro Ile Leu Glu	Ala Arg Gin A	sn Phe Ala Ty	r Gly Thr	
385	39 0	:	3 9 5		
Gln His Asp	Tyr Phe Asp H	is His Asn Ile	lie Gly Trp Th	r Arg Glu	
400	405	41	0	415	
Gly Asn Thi	Thr His Pro As	n Ser Gly Leu	Ala Thr lie M	et Ser Asp	
	420	425	430)	
Gly Pro Gly	Gly Glu Lys Tr	Met Tyr Val	Gly Gln Asn L	ys Ala Gly	
	435	440	445		
Gin Val Trp	His Asp Ile Thr	Gly Asn Lys	Pro Gly Thr Va	i Thr lie	
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Asn Ala As	Gly Trp Ala A	sn Phe Ser Va	al Asn Gly Gly	Ser Val Ser	
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lie Trp Val i	.ys Arg	•			
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His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp

AAPFNGTMMQ	YFEWYLPDDG	TLWTKVANEA	NNLSSLGITA	LWLPPAYKGT
SRSDVGYGVY	DLYDLGEFNO	KGAVRTKYGT	KAQYLQAIQA	
	AHAAGMQVYA			
DVVFDHKGGA	DGTEWVDAVE	VNPSDRNQE	SGTYQIQAWT	KFDFPGRGNT
YSSFKWRWYH	FDGVDWDESR	KLSRIYKFRG	IGKAWDWEVE	
TENGNYDYLM				
YADLDMDHPE	VVTELKSWGK	WYVNTTNIDG	FRLDAVKHIK	FSFFPDWLSD
VRSOTGKPLF -	TVGEYWSYD	NKLHNYIMKT	NGTMSLFDAP	LHNKFYTASK
SGGTFDMRTL	MTNTLMKDOP	TLAVTFVDNH	DTEPGOALOS	
	WVDPWFKPLA			
YAFILTROEG	YPCVFYGDYY	GIPOYNIPSL	KSKIDPLLIA	RRDYAYGTOH
DYLDHSDIIG	WTREGVTEKP	GSGLAALITD	GPGGSKWMY	V
GKOHAGKVFY				
DLTGNRSDTV	TINSDGWGEF	KVNGGSVSVW	VPRKTTVSTI	AWSITTRPWT
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 23 October 1997 (23.10.97)
 US

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(54) Title: MULTIPLY-SUBSTITUTED PROTEASE VARIANT AND AMYLASE VARIANT-CONTAINING CLEANING COMPOSITIONS

(57) Abstract

The present invention relates to cleaning compositions comprising a protease variant. One cleaning composition comprises a protease variant including a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of *Bacillus amyloliquefaciens* subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin; wherein when said protease variant includes a substitution of amino acid residue at one or more amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of *Bacillus amyloliquefaciens* subtilisin; and one or more cleaning adjunct materials. Another cleaning composition comprises a protease variant including a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin; an amylase variant and one or more cleaning adjunct materials. Methods for using the cleaning compositions are also provided.

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MULTIPLY-SUBSTITUTED PROTEASE VARIANT AND AMYLASE VARIANT-CONTAINING CLEANING COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates to cleaning compositions which comprise one or more protease enzymes which are multiply-substituted protease variants and one or more amylase enzymes which are amylase variants. More particularly, the present invention relates to laundry detergent compositions, dishwashing detergent compositions, hard surface cleaning compositions and personal cleansing compositions which comprise one or more multiply-substituted protease variants and one or more amylase variants.

BACKGROUND OF THE INVENTION

Various types of enzymes have long been used in laundry detergents to assist in the removal of certain stains from fabrics. Each class of enzyme (amylase, protease, etc.) generally catalyzes a different chemical reaction. For example, protease enzymes are known for their ability to hydrolyze (break down a compound into two or more simpler compounds) other proteins. This ability has been taken advantage of through the incorporation of naturally occurring or engineered protease enzymes to laundry detergent compositions.

In recent years the use of enzymes has also been investigated for use in automatic dishwashing compositions. Unfortunately, many enzymes, such as many conventional protease enzymes, do not translate well into the wash environment. Specifically, thermal stability, pH stability, oxidative stability and substrate specificity need to be optimized to ensure satisfactory performance.

U.S. Patent No. RE 34,606 to Estell et al. discloses the modification of subtilisin amion acid residues corresponding to positions in *Bacillus amyloliquefaciens* subtilisin tyrosine -1, aspartate +32, asparagine +155, tyrosine +104, methionine +222, glycine +166, histidine +64, glycine +169, phenylalanine +189, serine +33, serine +221, tyrosine +217, glutamate +156 and alanine +152.

U.S. Patent No. 5,182,204 discloses the modification of the amino acid +224 residue in *Bacillus amyloliquefaciens* subtilisin and equivalent positions in other subtilisins which may be modified by way of substitution, insertion or deletion and which may be combined with modifications to the residues identified in U.S. Patent No. RE 34,606 to form useful subtilisin mutants or variants. U.S. Patent No. 5,182,204 further discloses the modification of many amino acid residues within subtilisin, including specifically +99, +101, +103, +107, +126, +128, +135, +197 and +204.

U.S. Patent No. 5,679,630 to Baeck et al. discloses cleaning compositions comprising a protease variant including substitutions of amino acid residues with other amino acid residues at positions corresponding to position 76 in combination with one or more of the following positions 99, 101, 103, 104, 107, 123, 27, 105, 109, 126, 128, 135, 156, 166, 195, 197, 204, 206, 210, 216, 217, 218, 222, 260, 265 and/or 274 of *Bacillus amyloliquefaciens* subtilisin, and one or more cleaning composition materials.

In addition to protease enzymes, amylase enzymes have been used for a variety of different purposes, the most important of which are starch liquefaction, textile desizing, starch modification in the paper and pulp industry, and for brewing and baking. A further use of amylases, which is becoming increasingly important, is the removal of starch containing soils and stains during the washing of fabrics, hard surfaces, and/or dishes.

WO 94/18314 (Genencor) published August 18, 1994, WO 94/02596 (Novo) published February 3, 1994, and WO 95/10603 (Novo) published April 20, 1995, describe cleaning compositions which incorporate mutant amylases.

Other amylases known for use in cleaning compositions include both α - and β -amylases. α -Amylases are known in the art and include those disclosed in U.S. Patent Nos. 5,003,257; EP 252 666; WO 91/00353; FR 2,676,456; EP 285 123; EP 525 610; EP 368 341; and British Patent Specification No. 1,296,839 (Novo).

WO 95/26397 (Novo) published October 5, 1995 discloses an α -amylase having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10.

WO 98/05748 (P&G) published February 12, 1998 discloses variants of the α -amylases described in WO 95/26397 used in detergent compositions.

WO 98/30669 (Henkel) published July 16, 1998 discloses a protease and amylase-containing detergent composition wherein the protease is a protease mutant in which the amino acid leucine present in position 211 (BLAP counting method) in the wild-type protease is exchanged at this location for an aspartic acid or glutamic acid, and the amylase is an amylae mutant in which at least one methionine, tryptophan, cysteine or tyrosine present in the wild-type amylase is removed or exchanged for another amino acid which is in particular not cysteine or methionine. Examples of amylase mutants suitable for use in

the compositions of WO 98/30669 are disclosed in WO 94/02597 (Novo), WO 95/10603 (Novo) and WO 94/18314 (Genencor) and are commercially available as Duramyl[®] (Novo) and Purafect OxAm[®] (Genencor).

However, there continues to exist a consumer need for cleaning compositions that provide more enhanced and/or improved cleaning (removal and/or reduction) of soils and/or stains from substrates over conventional enzyme-containing cleaning compositions

By the present invention, it has been found that the combination of novel protease enzymes which are multiply-substituted protease variants and amylase enzymes which are amylase variants, especially α -amylase variants, provide enhanced and/or improved soil and/or stain removal benefits over conventional enzyme-containing cleaning compositions and/or over cleaning compositions containing the novel protease enzymes of the present invention in the absence of the amylase enzymes of the present invention.

Further, it has been surprisingly found that cleaning compositions comprising the novel combination of the novel protease enzymes of the present invention with the amylase enzymes of the present invention provide superior cleaning benefits over the cumulative cleaning benefits provided by cleaning compositions comprising one or the other, but not both, of the novel protease enzymes of the present invention or the amylase enzymes of the present invention.

Accordingly, it is an object of the present invention to provide cleaning compositions, especially laundry detergent compositions and/or dishwashing detergent compositions, having improved soil and/or stain removal benefits and/or fabric cleaning benefits.

Further, the specific combinations claimed in the present application are not identified in any of these prior art references.

SUMMARY OF THE INVENTION

The present invention meets the aforementioned needs in that it has been surprisingly discovered that the multiply-substituted protease variants of the present invention, when used in combination with the amylase variants of the present invention in cleaning compositions provide improved and enhanced cleaning ability, including, but not limited to, stain and/or soil removal and/or reduction and/or whiteness maintenance and/or dingy cleanup and/or spot and/or film removal and/or reduction, over conventional enzyme-containing cleaning compositions.

The multiply-substituted protease variants and amylase variants of the present invention are suitable for use in high and low density granular, heavy duty and light duty liquids, tablets, as well as synthetic detergent bar compositions, and other cleaning compositions.

In one aspect of the present invention a cleaning composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is selected from the group consisting of:
- (i) α -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas® α -amylase activity assay and/or;
- (ii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv) α-amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-

Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an α -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;

- (v) α -amylase according to (i-iv) wherein the α -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi) α -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii) α -amylase showing positive immunological cross-reactivity with antibodies raised against an α -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent α -amylase, wherein the parent α -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an α -amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an α amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent α-amylase has been deleted; and/or (B) at least one amino acid residue of said parent \alpha-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α -amylase; said variant having an α -amylase activity and exhibiting at least one of the following properties relative to said parent α -amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or α -amylolytic activity at neutral to relatively high pH values; increased α -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pl) so as to better match the pI value for α -amylase variant to the pH of the medium; and
 - (c) one or more cleaning adjunct materials.

In yet another aspect of the present invention, a fabric cleaning composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the fabric cleaning composition of a protease variant, wherein said protease variant is as described above;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above;
- (c) at least about 5% by weight of the fabric cleaning composition of a surfactant; and
 - (d) at least about 5% by weight of the fabric cleaning composition of a builder,

is provided.

In still another aspect of the present invention, a method for cleaning a fabric in need of cleaning comprising contacting the fabric with the fabric cleaning composition of the present invention is provided.

In still yet another aspect of the present invention, a dishwashing composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the dishwashing composition of a protease variant, wherein said protease variant is as described above;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above; and
- (c) from about 0.1% to about 10% by weight of a surfactant, is provided.

In still yet another aspect of the present invention, a method for cleaning a dish in need of cleaning comprising contacting the dish with the dishwashing composition of the present invention is provided.

In still yet another aspect of the present invention, a personal cleansing composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.001% to about 5% by weight of the personal cleansing composition of a protease variant, wherein said protease variant is as described above;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above; and
- (c) from about 0.1% to about 95% by weight of the personal cleansing composition of a surfactant system; and
- (d) optionally, from about 0.05% to about 50% by weight of the personal cleansing composition of an enzyme stabilizer, is provided.

In still yet another aspect of the present invention, a method for personal cleansing of a part of the human or lower animal body in need of cleansing comprising contacting the part with the personal cleansing composition of the present invention is provided.

In still yet another aspect of the present invention, a cleaning composition comprising:

(a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of a

protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;

- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is selected from the group consisting of:
- (i) α -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas® α -amylase activity assay and/or;
- (ii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv) α -amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an α -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;
- (v) α -amylase according to (i-iv) wherein the α -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi) α -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii) α -amylase showing positive immunological cross-reactivity with antibodies raised against an α -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent α -amylase, wherein the parent α -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an α -amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an α -amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent α -amylase has been deleted; and/or (B) at least one amino acid residue of said parent α -amylase has been replaced by a different amino acid

residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α -amylase; said variant having an α -amylase activity and exhibiting at least one of the following properties relative to said parent α -amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or α -amylolytic activity at neutral to relatively high pH values; increased α -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for α -amylase variant to the pH of the medium; and

(c) one or more cleaning adjunct materials, is provided.

In still yet another aspect of the present invention, a fabric cleaning composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the fabric cleaning composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above;
- (c) at least about 5% by weight of the fabric cleaning composition, of a surfactant; and
- (d) at least about 5% by weight of the fabric cleaning composition, of a builder, is provided.

In still another aspect of the present invention, a method for cleaning a fabric in need of cleaning comprising contacting the fabric with the fabric cleaning composition of the present invention is provided.

In still yet another aspect of the present invention, a dishwashing composition comprising:

(a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.0001% to about 10% by weight of the fabric cleaning composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;

- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above; and
- (c) from about 0.1% to about 10% by weight of the dishwashing composition, of a surfactant, is provided.

In still yet another aspect of the present invention, a method for cleaning a dish in need of cleaning comprising contacting the dish with the dishwashing composition of the present invention is provided.

In still yet another aspect of the present invention, a personal cleansing composition comprising:

- (a) a protease variant, preferably an effective amount of a protease variant, more preferably from about 0.001% to about 5% by weight of the personal cleansing composition of a protease variant, wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;
- (b) an amylase variant, preferably an effective amount of an amylase variant, more preferably from about 0.0001% to about 10% by weight of the cleaning composition of an amylase variant, wherein said amylase variant is as described above; and
- (c) from about 0.1% to about 95% by weight of the personal cleansing composition, of a surfactant system; and
- (d) optionally, from about 0.05% to about 50% by weight of the personal cleansing composition, of an enzyme stabilizer, is provided.

In still yet another aspect of the present invention, a method for personal cleansing of a part of the human or lower animal body in need of cleansing comprising contacting the part with the personal cleansing composition of the present invention is provided.

Accordingly, it is an object of the present invention to provide cleaning compositions having a combination of a protease variant and amylase variant capable of providing improved and enhanced cleaning of fabrics, dishware, tableware, kitchenware, cookware and other hard surface substrates. It is a further object of the present invention to provide methods for fabric, dishware, tableware, kitchenware, cookware and other hard surface substrate cleansing via the use of the protease variant/amylase variant-containing cleaning compositions of the present invention.

These and other objects, features and advantages will be clear from the following detailed description, examples and appended claims.

All percentages, ratios and proportions herein are on a weight basis unless otherwise indicated. All documents cited herein are hereby incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 A-C depict the DNA and amino acid sequence for *Bacillus* amyloliquefaciens subtilisin and a partial restriction map of this gene.

Fig. 2 depicts the conserved amino acid residues among subtilisins from *Bacillus* amyloliquefaciens (BPN)' and *Bacillus lentus* (wild-type).

Figs. 3A and 3B depict the amino acid sequence of four subtilisins. The top line represents the amino acid sequence of subtilisin from *Bacillus amyloliquefaciens* subtilisin (also sometimes referred to as subtilisin BPN'). The second line depicts the amino acid sequence of subtilisin from *Bacillus subtilis*. The third line depicts the amino acid sequence of subtilisin from *B. licheniformis*. The fourth line depicts the amino acid sequence of subtilisin from *Bacillus lentus* (also referred to as subtilisin 309 in PCT WO89/06276). The symbol * denotes the absence of specific amino acid residues as compared to subtilisin BPN'.

DETAILED DESCRIPTION OF THE INVENTION

l. <u>Proteases</u> - Proteases are carbonyl hydrolases which generally act to cleave peptide bonds of proteins or peptides. As used herein, "protease" means a naturally occurring protease or recombinant protease. Naturally-occurring proteases include α -aminoacylpeptide hydrolase, peptidylamino acid hydrolase, acylamino hydrolase, serine carboxypeptidase, metallocarboxypeptidase, thiol proteinase, carboxylproteinase and metalloproteinase. Serine, metallo, thiol and acid protease are included, as well as endo and exo-proteases.

The present invention includes protease enzymes which are non-naturally occurring carbonyl hydrolase variants (protease variants) having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Specifically, such protease variants have an amino acid sequence not found in nature, which is derived by replacement of a plurality of amino acid residues of a precursor protease with different amino acids. The precursor protease may be a naturally-occurring protease or recombinant protease. As stated earlier, the protease variants are designed to have trypsin-like specificity and preferably also be bleach stable.

The protease variants useful herein encompass the substitution of any of the nineteen naturally occurring L-amino acids at the designated amino acid residue positions.

Such substitutions can be made in any precursor subtilisin (procaryotic, eucaryotic, mammalian, etc.). Throughout this application reference is made to various amino acids by way of common one- and three-letter codes. Such codes are identified in Dale, M.W. (1989), Molecular Genetics of Bacteria, John Wiley & Sons, Ltd., Appendix B.

The protease variants useful herein are preferably derived from a *Bacillus* subtilisin. More preferably, the protease variants are derived from *Bacillus* lentus subtilisin and/or subtilisin 309.

<u>Carbonyl Hydrolases</u> - Carbonyl hydrolases are protease enzymes which hydrolyze compounds containing

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bonds in which X is oxygen or nitrogen. They include naturally-occurring carbonyl hydrolases and recombinant carbonyl hydrolases. Naturally-occurring carbonyl hydrolases principally include hydrolases, e.g., peptide hydrolases such as subtilisins or metalloproteases. Peptide hydrolases include α-aminoacylpeptide hydrolase, peptidylamino acid hydrolase, acylamino hydrolase, serine carboxypeptidase, metallocarboxypeptidase, thiol proteinase, carboxylproteinase and metalloproteinase. Serine, metallo, thiol and acid protease's are included, as well as endo and exo-proteases.

Subtilisins - Subtilisins are bacterial or fungal proteases which generally act to cleave peptide bonds of proteins or peptides. As used herein, "subtilisin" means a naturally-occurring subtilisin or a recombinant subtilisin. A series of naturally-occurring subtilisins is known to be produced and often secreted by various microbial species. Amino acid sequences of the members of this series are not entirely homologous. However, the subtilisins in this series exhibit the same or similar type of proteolytic activity. This class of serine proteases share a common amino acid sequence defining a catalytic triad which distinguishes them from the chymotrypsin related class of serine proteases. The subtilisins and chymotrypsin related serine proteases both have a catalytic triad comprising aspartate, histidine and serine. In the subtilisin related proteases the relative order of these amino acids, reading from amino to carboxy terminus, is aspartatehistidine-serine. In the chymotrypsin related proteases, the relative order, however, is histidine-aspartate-serine. Thus, subtilisin herein refers to a serine protease having the catalytic triad of subtilisin related proteases. Examples include, but are not limited to, the subtilisins identified in Fig. 3 herein. Generally, and for purposes of the present invention, numbering of the amino acids in proteases corresponds to the numbers assigned to the mature Bacillus amyloliquefaciens subtilisin sequence presented in Fig. 1.

Protease Variants - A "protease variant" has an amino acid sequence which is derived from the amino acid sequence of a "precursor protease." The precursor proteases include naturally-occurring proteases and recombinant proteases. The amino acid sequence of the protease variant is "derived" from the precursor protease amino acid sequence by substitution, deletion or insertion of one or more amino acids of the precursor amino acid sequence. Such modification is of the "precursor DNA sequence" which encodes the amino acid sequence of the precursor protease rather than manipulation of the precursor protease enzyme per se. Suitable methods for such manipulation of the precursor DNA sequence include methods disclosed herein, as well as methods know to those skilled in the art (see, for example, EP 0 328 299, WO 89/06279 and the U.S. patents and applications already referenced herein).

In a preferred embodiment, the protease variants which are protease enzymes useful in the present invention cleaning compositions comprise protease variants including a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin; and one or more cleaning adjunct materials.

While any combination of the above listed amino acid substitutions may be employed, the preferred protease variant enzymes useful for the present invention comprise the substitution, deletion or insertion of amino acid residues in the following combinations:

(1) a protease variant including substitutions of the amino acid residues at position 103 and at one or more of the following positions 236 and 245;

- (2) a protease variant including substitutions of the amino acid residues at positions 103 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 248, 252, 257, 260, 270 and 275;
- (3) a protease variant including substitutions of the amino acid residues at positions 103 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 170, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 222, 230, 232, 248, 252, 257, 260, 261, 270 and 275; and
- (4) a protease variant including substitutions of the amino acid residues at positions 103, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 243, 248, 252, 257, 260, 270 and 275.

A more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table I) selected from the group consisting of:

Table I

	1	Τ	т	т	T			·				
76	98	103	104							 		
76	78	103	104		 	 					-	
76	103	104	107			 	 	<u> </u>	 -			
4	76	103	104	ļ		-	 			<u> </u>	<u> </u>	ļ <u>.</u>
76	103	104	246	<u> </u>	-	<u> </u>						<u> </u>
76	77	103	104			ļ						
						<u> </u>						
76	103	104	183	218								
16	76	103	104	248								
1	76	103	104				 					
76	103	104	261				 					ļ——
76	103	104	160			 -						
76	103	104	216									
17	76	103	104									
				· 								
37	76	103	104							_		
76	77	103	104	174								
38	76	103	104									
		i				<u> </u>						i i

38	76	103	104	237	T	-	T	1		1	1	
8	76	103	104	 	-	+	+	-		-		
76	103	104	183		 	-	-	 			<u> </u>	
19	76	103	104		 	 		 	 	 		ļ
13	76	103	104	+	 		 	+	┼	 	-	
19	76	103	104	 	 	-	-		-	 		
76	103	104	184	 	 	 	 	 		-		
76	103	104	252	1	 	+-		 		 	 	
76	103	104	259						 			
76	103	104	251			 			 		<u> </u>	
76	86	103	104	 		-				<u> · </u>		
72	76	103	104	185			 	<u> </u>		<u> </u>		
76	103	104	237	274		 	 					
76	103	104	160									
76	103	104	228									
55	76	103	104	240		<u> </u>						
76	103	104	254			1						
76	103	104	204									
76	103	104	204									
43	76	103	104		7,							
76	103	104	159									
10	76	103	104	177								
58	76	103	104									
76	103	104	270									
76	103	104	185									
27	76	103	104									
76	103	104	262									
76	78	103	104						— ——			
24	76	103	104									
76	103	104	166	236	251							
17	76	103	104	237								
76	103	104	130									
							<u>-</u>					

76	103	104	109	1				1	Τ	T	Τ	Γ
76	99	103	104	204	ļ	 			 -			
76	103	104	181	-							ļ	
12	76	103	104	 -				 				
76	103	104	212	271	 	 		 		 -	-	
76	103	104	252	261		 						
76	103	104	242			 	 	<u> </u>				
76	103	104	271			 			-			
12	76	103	104	242								
43	76	103	104	116	183	 						
76	103	104	258			 	 					
76	103	104	271					-				
61	76	103	104	<u> </u>								
38	76	103	104	182	263			<u> </u>				
76	103	104	182	272		<u> </u>						
76	103	104	109	246								
76	87	103	104	206	249	265						
76	103	104	137	238	271							
103	104	228										
76	103	104	182	198								
21	76	103	104	182								
76	103	104	119	137								
76	103	104	137	248								
13	76	103	104	206								
76	103	104	206									
76	103	104	212	258								
58	76	103	104	271								
76	103	104	206	261								
4	76	103	104	206								
76	77	103	104	206								
76	103	104	158									
76	103	104	206									
								ــــــــــــــــــــــــــــــــــــــ		i		

4	76	103	104	159	217	251						
4	76	103	104	159	217	252	-					
76	77	103	104	133	185	251			-			
76	103	104	159	206	244	 	-					
4	76	103	104	188	+	 						
4	76	103	104	158		 	-					
76	77	103	104	185	 	 	 		-			
76	103	104	206	251		 	 		_		_	
48	76	103	104	111	159	 	+	-				
68	76	103	104	159	236	 			+			
42	76	103	104	159			-				_	
12	62	76	103	104	159		<u> </u>	-	+-	+-		
42	76	103	104	159			-	+		_		
76	103	104	146	159			†					
76	103	104	159	238				 	-		-	+
76	103	104	159	224			 	+				
76	103	104	212	268	271			†			+	
76	89	103	104					†			+	
76	87	103	104	212	271			1	1-	_		
76	103	104	212	245	271				1			1
76	103	104	134	141	212	271		1	 			
76	103	104	212	236	243	271						
76	103	104	109	245							_	
76	103	104	109	210						1		
20	62	76	103	104								
68	76	103	104	236								
68	76	103	104	159	236	271					 	
68	76	103	104	159	236	245						
68	76	103	104	159	217	236	271					
17	68	76	103	104						-		
68	76	103	104							1		
68	76	103	104	159	236					1-		
								<u> </u>	Ц			

68	3 75	76	10:	3 104	159	1 226	-						
68													
12			L			159		5 245	<u>'</u>				
68	L_					236							
68						236							
68						184							
68						243	_						
68					236	245							
<u>L</u>					159	<u> </u>							
68					159	236	249	'					
68					236	249				1			
76				_1							_		
12		103		222	249		1		 	\dashv	_		
76	103	104	173	222						+-	-		
76	103	104	222	263				+	+-				
21	76	103	104	222	237	263	 	-	-		-+		
76	103	104	109	222			 	-	-	+-			
76	103	104	109	222	271		 -			+-			
61	76	103	104	222			 -			-			
76	103	104	137	222			_	+		+-	-+		
76	103	104	109	222	248						\dashv		
76	103	104	222	249				-		+	-		
68	76	103	104	159	236	245	261	 	<u> </u>	+	-		
68	76	103	104	141	159	236	245	255	 -		+		
68	76	103	104	159	236	245	247	 		-	_		
68	76	103	104	159	174	204	236	245		-			
68	76	103	104	159	204	236	245	 		-	_		
68	76	103	104	133	159	218	236	245		-			
68	76	103	104	159	232	236	245			+	+		
68	76	103	104	159	194	203	236	245		+-	+	_	
12	76	103	104	222	245				 :		+	_	
76	103	104	232	245	-					-	+		
24	68	76	103	104	159	232	236	245		 	-		
								_ , •	 -	<u> </u>			

68 76 103 104 159 213 232 236 245 260 12 76 103 104 222 244 245 12 76 103 222 210 245 12 76 103 104 130 222 245 22 68 76 103 104 184 68 76 103 104 184 68 103 104 159 232 236 245 252 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 <th></th>	
12 76 103 222 210 245 12 76 103 104 130 222 245 22 68 76 103 104 184 68 76 103 104 184 252 68 103 104 159 232 236 245 252 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 68 87 103 104 159 232 236 245 252 12 76 103 104 130 222 245 248 262 12 <td></td>	
12 76 103 104 130 222 245 22 68 76 103 104 184 68 76 103 104 184 103 68 103 104 159 232 236 245 248 252 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 68 87 103 104 159 232 236 245 252 12 76 103 104 130 222 245 248 262 12 76 103 104 130 222 227 </td <td></td>	
22 68 76 103 104 <	
68 76 103 104 184	
68 103 104 159 232 236 245 248 252 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 68 87 103 104 159 232 236 245 252 12 76 103 104 130 222 245 248 262 12 76 103 104 130 222 245 262 12 76 103 104 130 222 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245 261	
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68 103 104 140 159 232 236 245 252 43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 252 68 87 103 104 159 232 236 245 252 12 76 103 104 130 222 245 248 262 12 76 103 104 130 215 222 245 262 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245 261 76 103 104 130 222 245	ŀ
43 68 103 104 159 232 236 245 252 43 68 103 104 159 232 236 245 43 68 103 104 159 232 236 245 252 68 87 103 104 159 232 236 245 252 275 12 76 103 104 130 222 245 248 262 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245 261 76 103 104 130 222 245 261	
43 68 103 104 159 232 236 245 43 68 103 104 159 232 236 245 252 68 87 103 104 159 232 236 245 252 275 12 76 103 104 130 222 245 248 262 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245 261	
43 68 103 104 159 232 236 245 252 68 87 103 104 159 232 236 245 252 275 12 76 103 104 130 222 245 248 262 12 76 103 104 130 215 222 245 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245 261	
68 87 103 104 159 232 236 245 252 275 12 76 103 104 130 222 245 248 262 12 76 103 104 130 215 222 245 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245	
12 76 103 104 130 222 245 248 262 12 76 103 104 130 215 222 245 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245	
12 76 103 104 130 215 222 245 12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245	
12 76 103 104 130 222 227 245 262 12 76 103 104 130 222 245 261 76 103 104 130 222 245	
12 76 103 104 130 222 245 261 76 103 104 130 222 245	
76 103 104 130 222 245	
12 76 103 104 130 218 222 245 262 269	
12 57 76 103 104 130 222 245 251	
12 76 103 104 130 170 185 222 243 245	
12 76 103 104 130 222 245 268	
12 76 103 104 130 222 210 245	
68 103 104 159 232 236 245 257	
68 103 104 116 159 232 236 245	
68 103 104 159 232 236 245 248	
10 68 103 104 159 232 236 245	
68 103 104 159 203 232 236 245	
68 103 104 159 232 236 237 245	
68 76 79 103 104 159 232 236 245	
68 103 104 159 183 232 236 245	ı

68	3 103	3 104	1 159	174	206	1 222	1 000	1 046						
68						232			<u>'</u>					
						236								
68					232	236	245				_			_
68		103		159	232	236	245	1		+-				
68	103	104	159	215	232	236	245		+	+				
68	103	104	159	232	236	245	248		-	+	+-			
68	76	103	104	159	232	236	245		+	-	\dashv			
68	76	103	104	159	210	232	236	245	+	+-	+			
68	76	103	104	159	232	236	245	257		+-				
76	103	104	232	236	245	257		 	 	-	-			_
68	103	104	159	232	236	245	257	275	+	+-	-			-
76	103	104	257	275				 -	+	-	+-		 -	\dashv
68	103	104	159	224	232	236	245	257		┼				\dashv
76	103	104	159	232	236	245	257			+				\dashv
68	76	103	104	159	209	232	236	245	 	┼	+-			\dashv
68	76	103	104	159	211	232	236	245	 	 				\dashv
12	68	76	103	104	159	214	232	236	245	┼	-			\dashv
68	76	103	104	159	215	232	236	245	-	-		-+		\dashv
12	68	76	103	104	159	232	236	245	 	 		\dashv	 -	\dashv
20	68	76	103	104	159	232	236	245	259	-	+-	+		\dashv
68	8.7	76	103	104	159	232	236	245	260	ļ	+-	+		\dashv
68	76	103	104	159	232	236	245	261			+-	+		+
76	103	104	232	236	242	245			 					$\frac{1}{2}$
68	76	103	104	159	210	232	236	245			╁	_		$\frac{1}{2}$
12	48	68	76	103	104	159	232	236	245		+			\dashv
76	103	104	232	236	245						 	_		$\frac{1}{2}$
76	103	104	159	192	232	236	245				+	+		$\frac{1}{2}$
76	103	104	147	159	232	236	245	248	251		+-	+		-
12	68	76	103	104	159	232	236	245	272		+-	+		-
68	76	103	104	159	183	206	232	236	245		-	-		-
68	76	103	104	159	232	236	245	256	-		-			-
68	76	103	104	159	206	232	236	245			 	+		-
						i					<u> </u>			

68	76		ŀ	i	159		236					
	, 0	103	104	116	159	170	185	245	236	245	 	
61	68	103	104	159	232	236	245	248	252	213	 	
43	68	103	104	159	232	236	245	248	252	-	 	
68	103	104	159	212	232	236	245	248	252		-	
68	103	104	99	159	184	232	236	245	248	252	 	
103	104	159	232	236	245	248	252	-			 	
68	103	104	159	209	232	236	245	248	252		_	
68	103	104	109	159	232	236	245	248	252	 -		
20	68	103	104	159	232	236	245	248	252			ļ
68	103	104	159	209	232	236	245	248	252			
68	103	104	159	232	236	245	248	252	261			
68	103	104	159	185	232	236	245	248	252			
68	103	104	159	210	232	236	245	248	252	<u> </u>		
68	103	104	159	185	210	232	236	245	248	252		
68	103	104	159	212	232	236	245	248	252			
68	103	104	159	213	232	236	245	248	252			<u></u>
68	103	104	213	232	236	245	248	252				
68	103	104	159	215	232	236	245	248	252			
68	103	104	159	216	232	236	245	248	252			
20	68	103	104	159	232	236	245	248	252			
	103	104	159	173	232	236	245	248	252			
	103	104	159	232	236	245	248	251	252			
	103	104	159	206	232	236	245	248	252			
	103	104	159	232	236	245	248	252				
	68	103	104	159	232	236	245	248	252	_		
	103	104	159	232	236	245	248	252	255			
	103	104	159	232	236	245	248	252	256			
	103	104	159	232	236	245	248	252	260			
	103	104	159	232	236	245	248	252	257			
	103	104	159	232	236	245	248	252	258			
8	68	103	104	159	232	236	245	248	252	269		

68	103	104	116	159	232	236	245	248	252	260	Τ	Ι
68	103	104	159	232	236	245	248	252	261	 		
68	103	104	159	232	236	245	248	252	261			
68	76	103	104	159	232	236	245	248	252		ļ	
68	103	104	232	236	245	248	252	 	 			
103	104	159	232	236	245	248	252					
68	103	104	159	232	236	245	248	252				
18	68	103	104	159	232	236	245	248	252			
68	103	104	159	232	236	245	248	252				
68	76	101	103	104	159	213	218	232	236	245	260	
68	103	104	159	228	232	236	245	248	252	· ·		
33	68	76	103	104	159	232	236	245	248	252		
68	76	89	103	104	159	210	213	232	236	245	260	
61	68	76	103	104	159	232	236	245	248	252		
103	104	159	205	210	232	236	245					
61	68	103	104	130	159	232	236	245	248	252		
61	68	103	104	133	137	159	232	236	245	248	252	
61	103	104	133	159	232	236	245	248	252			
68	103	104	159	232	236	245	248	252				
68	103	104	159	218	232	236	245	248	252			
61	68	103	104	159	160	232	236	245	248	252		
3	61	68	76	103	104	232	236	245	248	252		
61	68	103	104	159	167	232	236	245	248	252		
97	103	104	159	232	236	245	248	252				
98	103	104	159	232	236	245	248	252				
99	103	104	159	232	236	245	248	252				
101	103	104	159	232	236	245	248	252				
102	103	104	159	232	236	245	248	252				
103	104	106	159	232	236	245	248	252				
103	104	109	159	232	236	245	248	252				
103	104	159	232	236	245	248	252	261				
62	103	104	159	232	236	245	248	252				

103	104	159	184	232	236	245	248	252		Г	T	
103	104	159	166	232	236	245	248	252			 	
103	104	159	217	232	236	245	248	252	<u> </u>		 	
20	62	103	104	159	213	232	236	245	248	252		
62	103	104	159	213	232	236	245	248	252			
103	104	159	206	217	232	236	245	248	252			
62	103	104	159	206	232	236	245	248	252		 	
103	104	130	159	232	236	245	248	252				
103	104	131	159	232	236	245	248	252				
27	103	104	159	232	236	245	248	252				
38	103	104	159	232	236	245	248	252				
38	76	103	104	159	213	232	236	245	260		<u></u>	
68	76	103	104	159	213	232	236	245	260	271		
68	76	103	104	159	209	213	232	236	245	260		
68	76	103	104	159	210	213	232	236	245	260		
68	76	103	104	159	205	213	232	236	245	260		
68	76	103	104	159	210	232	236	245	260			
68	103	104	159	213	232	236	245	260	-			
76	103	104	159	213	232	236	245	260				
68	103	104	159	209	232	236	245					
68	103	104	159	210	232	236	245					
68	103	104	159	230	232	236	245					
68	103	104	159	126	232	236	245					
68	103	104	159	205	232	236	245					
68	103	104	159	210	232	236	245					
103	104	159	230	236	245							
68	103	104	159	232	236	245	260					
103	104	159	232	236	245							
68	103	104	159	174	232	236	245	257				
68	103	104	159	194	232	236	245	257				
68	103	104	159	209	232	236	245	257				
103	104	159	232	236	245	257						

103 104 159 213 232 236 245 260 103 104 159 210 232 236 245 248 23 103 104 159 209 232 236 245 257 68 76 103 104 159 210 213 232 236 12 103 104 159 209 213 232 236 24 103 104 209 232 236 245 257 257 103 104 159 205 210 213 232 236 24 103 104 159 205 210 213 232 236 24	52 36 245 45 260 45 260 60 36 245	260
103 104 159 210 232 236 245 248 23 103 104 159 209 232 236 245 257 68 76 103 104 159 210 213 232 236 12 103 104 159 209 213 232 236 24 103 104 209 232 236 245 257 103 104 159 205 210 213 232 236 24 103 104 159 205 210 213 232 236 24	36 245 45 260 45 260 60	260
103 104 159 209 232 236 245 257 68 76 103 104 159 210 213 232 23 12 103 104 159 209 213 232 236 24 103 104 209 232 236 245 257 103 104 159 205 210 213 232 236 24 103 104 159 205 210 213 232 236 24	36 245 45 260 45 260 60	260
68 76 103 104 159 210 213 232 23 12 103 104 159 209 213 232 236 24 103 104 209 232 236 245 257 103 104 159 205 210 213 232 236 24	45 260 45 260 60	260
12 103 104 159 209 213 232 236 24 103 104 209 232 236 245 257 257 103 104 159 205 210 213 232 236 24	45 260 45 260 60	260
103 104 209 232 236 245 257 103 104 159 205 210 213 232 236 24	45 260 60	
103 104 159 205 210 213 232 236 24	60	
102 104 150 005 000 000	60	
103 104 159 205 209 232 236 245 26		
	36 245	
68 103 104 159 205 209 210 232 23		
103 104 159 205 209 210 232 236 24	45 257	
103 104 159 205 209 232 236 245 25	57	
68 103 104 159 205 209 210 232 23	36 245	260
103 104 159 205 209 210 232 236 24	45	
103 104 159 209 210 232 236 245		
103 104 159 205 210 232 236 245		
68 103 104 128 159 232 236 245		
48 103 104 159 230 236 245		
48 68 103 104 159 209 232 236 24	45	
48 68 103 104 159 232 236 245 24	48 252	
48 68 103 104 159 232 236 245 25	57 261	
102 103 104 159 212 232 236 245 24	18 252	
12 102 103 104 159 212 232 236 24	15 248	252
101 102 103 104 159 212 232 236 24	15 248	252
98 102 103 104 159 212 232 236 24	15 248	252
102 103 104 159 213 232 236 245 24	18 252	
103 104 131 159 232 236 245 248 25	52	
103 104 159 184 232 236 245 248 25	52	
103 104 159 232 236 244 245 248 25	52	
62 103 104 159 213 232 236 245 24	8 252	256
12 62 103 104 159 213 232 236 24	15 248	252

101	103	104	159	105	1 222	226	7 245			,	т	
			<u> </u>	185	232	236	245	248	252			
101	103	104	159	206	232	236	245	248	252			
101	103	104	159	213	232	236	245	248	252			
98	102	1.03	104	159	232	236	245	248	252			
101	102	103	104	159	232	236	245	248	252			
98	102	103	104	159	212	232	236	245	248	252		
98	102	103	104	159	212	232	236	248	252			
62	103	104	109	159	213	232	236	245	248	252		
62	103	104	159	212	213	232	236	245	248	252		
62	101	103	104	159	212	213	232	236	245	248	252	
103	104	159	232	245	248	252						
103	104	159	230	245								
62	103	104	130	159	213	232	236	245	248	252		
101	103	104	130	159	232	236	245	248	252			
101	103	104	128	159	232	236	245	248	252			
62	101	103	104	159	213	232	236	245	248	252		
62	103	104	128	159	213	232	236	245	248	252		
62	103	104	128	159	213	232	236	245	248	252		
101	103	104	159	232	236	245	248	252	260			
101	103	104	131	159	232	236	245	248	252	 -		
98	101	103	104	159	232	236	245	248	252			
99	101	103	104	159	232	236	245	248	252			
101	103	104	159	212	232	236	245	248	252			
76	103	104	167	170	194							
101	103	104	159	209	232	236	245	248	252			
101	103	104	159	210	232	236	245	248	252			
101	103	104	159	205	232	236	245	248	252			
101	103	104	159	230	236	245						
101	103	104	159	194	232	236	245	248	252			
76	101	103	104	159	194	232	236	245	248	252		
101	103	104	159	230	232	236	245	248	252			
62	103	104	159	185	206	213	232	236	245	248	252	271
			<u>_</u>	l			I					

An even more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table II) selected from the group consisting of:

Γ							Table	II			
-					_						
N76D	A98E	S103A	V104I								
N76D	S78T	S103A	V1041								
N76D	S103A	V104I	1107V								
V4E	N76D	S103A	V1041								_
N76D	S103A	V1041	1246V								
N76D	N77D	S103A	V1041								
N76D	S103A	V104I	N183D	N218I							
A16T	N76D	S103A	V104I	N248D							٦
AlE	N76D	S103A	V104I								7
N76D	S103A	V104I	N261D								7
N76D	S103A	V104I	S160T		ļ					1	1
N76D	S103A	V104I	S216C		<u> </u>					1	1
H17Q	N76D	S103A	V1041								1
S37T	N76D	S103A	V104I		<u> </u>						1
N76D	N77D	S103A	V104I	A174V							٦
T385	N76D	S103A	V104I	ļ							1
T385	N76D	S103A	V104I	K237Q		<u> </u>					1
18V	N76D	S103A	V1041	<u> </u>							7
N76D	S103A	V104I	N183D								7
R19L	N76D	S103A	V1041								1
A13V	N76D	S103A	V104I								1
R19C	N76D	S103A	V104I	<u> </u>							1
N76D	S103A	V104I	N184D								1
N76D	S103A	V1041	N252D								1
N76D	S103A	V104I	S259C								
N76D	S103A	V1041	K251T			<u> </u>					1
N76D	P86S	S103A	V1041								1
172V	N76D	S103A	V104I	N185D							1
N76D	S103A	V104I	K237E	T274A							
N76D	S103A	V104I	S160L								

							26							
N761	S103A	V1041	A2281	,									T	
P55S	N76D	S103A	V104I	S240T										1-
N76E	S103A	V1041	A254T											1-
N76D	S103A	1104N	N204T				1						 	+
N76D	S103A	V104I	N204D	,									 	
N43S	N76D	\$103A	V104I											
N76D	S103A	V1041	G159D							1			1	
RIOH	N76D	S103A	V104I	V177A						7				+
T58S	N76D	S103A	V1041							1				
N76D	S103A	V104I	A270V							†			 	
N76D	S103A	V104I	N185D							1			 	
K27N	N76D	S103A	V1041							1				
N76D	S103A	V104I	L262M											_
N76D	S78P	S103A	V1041			1		7	··					
S24P	N76D	S103A	V104I			7		7		1				
N76D	S103A	V104I	S166G	Q236R	K251R									
H17L	N76D	S103A	V1041	K237E				1			1			
N76D	S103A	V1041	S130L								7			
N76D	S103A	V104I	Q109R								1			
N76D	S99R	S103A	V1041	N204T				1			\top		·	
N76D	S103A	V104I	D181N					1			1			
Q12R	N76D	S103A	V104I					\top			1		· · · · · · · · · · · · · · · · · · ·	
N76D	S103A	V104I	S212P	E271 V							\top			
N76D	S103A	V104I	N252K	N261Y							7			
N76D	S103A	V104I	S242T					1			\dagger			
N76D	S103A	V1041	E271Q					\top			1			
Q12R	N76D	S103A	V104I	S242T							\top			
N43S	N76D	S103A	V104I	N116K	N183I						1			
N76D	S103A	V104I	G258R							_	\top			
N76D	S103A	V104I	E271G					1			\top			, <u>. </u>
G61R	N76D	S103A	V104I					1			\dagger			
T38S	N76D	S103A		Q182R	Y263H			+			+	$\neg \uparrow$		
N76D	S103A			A272S				\top			+	\dashv		
	\$103A			1246V				\dagger			\dagger			
					H249Q	S265G		+			\dagger	_		
					E271V	22030		+			\dagger	+		
			Z / 10		/ I V			_Ļ_						

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						2	, 		-				
S103.	A V1041	A2287	г 📗										_
N76E	S103A	V1041	Q182F	1198V									_
L21M	1 N76D	S103A	V104I	Q182R									_
N76D	S103A	V104I	M1191	Q137R									_
N76D	S103A	V104I	Q137R	N248S							1-		_
A13T	N76D	S103A	V1041	Q206R									_
N76D	S103A	V104I	Q206R										_
N76D	S103A	V104I	S212P	G258R									_
T58S	N76D	S103A	V104I	E271G									_
N76D	S103A	V1041	Q206E	N261D									_
V4E	N76D	S103A	V1041	Q206E									7
N76D	N77D	S103A	V104I	Q206E					1			1	1
N76D	S103A	V1041	A158E									1	1
N76D	S103A	V1041	Q206E									 	1
V4E	N76D	S103A	V104I	G159D	L217E	K251Q						 	1
V4E	N76D	S103A	V104I	G159D	L217E	N252D						 	1
N76D	N77D	S103A	V1041	A133T	N185D	K251T			1			1	1
N76D	S103A	V1041	G159D	Q206E	V244A						†	1	1
V4E	N76D	S103A	V104I	S188E								1	1
V4E	N76D	S103A	V104I	A158E									1
N76D	N77D	S103A	V104I	N185D									1
N76D	S103A	V1041	Q206E	K251T									1
A48T	N76D	S103A	V1041	LIIIM	G159D								1
V68A	N76D	S103A	V104I	G159D	Q236H								l
1.42V	N76D	S103A	V104I	G159D									
Q12H	N62H	N76D	S103A	V1041	G159D								
L42I	N76D	S103A	V104I	G159D									
N76D	S103A	V1041	G146S	G159D									
N76D	S103A	V104I	G159D	N238S									
N76D	\$103A	V1041	G159D	T224A									
N76D	S103A	V104I	S212P	V268F	E271V								
N76D	E89A	S103A	V104I					,					
N76D	S87R	S103A	V104I	S212P	E271V								
N76D	S103A	V104I	S212P		E271V		_						
N76D	\$103A	V1041	T134S			E271V							
N76D	S103A					E271V							
									<u> </u>		L	نـــــــــــــــــــــــــــــــــــــ	

							8					
N761	S103/	A V1041	Q1091	R Q245R								
N761	S103	V1041	Q109F	R P210L	<u> </u>							_
G20\	/ N62S	N76D	S103A	V1041							1	
V68A	N76D	S103.A	V1041	Q236H					\Box		1	_
V68A	N76D	S103A	V104I	G159D	Q236H	E271	v				1	_
V68A	N76D	S103A	V104I	G159D	Q236H	Q245	R				1	_
V68A	N76D	S103A	V104I	G159D	L2171	Q236	H E271	,			1	_
H17Q	V68A	N76D	S103A	V104I							†	_
V68A	N76D	S103A	V104I								†	_
V68A	N76D	S103A	V104I	G159D	Q236R						1	_
V68A	L75R	N76D	S103A	V104I	G159D	Q236	н					_
V68A	N76D	N76D	S103A	A114V	V121I	G1591	Q2361	I Q245R		 		_
Q12R	V68A	N76D	S103A	V1041	G159D	Q2361	1					
V68A	N76D	S103A	V1041	G159D	Y209S	Q2361	1 T253K		\top			_
V68A	N76D	S103A	V104I	N117K	G159D	N1845	Q236H					
V68A	N76D	S103A	V1041	G159D	Q236H	N243I						7
V68A	N76D	S103A	V104I	G159D	Q236H	Q2451						1
V68A	N76D	S103A	V1041	A142V	G159D				7			1
V68A	N76D	S103A	V1041	N123S	G159D	Q236H	H249Y					1
V68A	N76D	S103A	V104I	G159D	Q236H	H249C						1
N76D	S103A	V1041	M222S	Q245R					\top			1
Q12R	N76D	S103A	V104I	M222S	H249R							1
N76D	S103A	V104I	N173R	M222S					T			1
N76D	S103A	V104I	M222S	Y263F								1
L21M	N76D	S103A	V1041	M222S	K237R	Y263F						1
N76D	S103A	V104I	Q109R	M222S								1
N76D	S103A	V104I	Q109R	M222S	E271D							ĺ
G61R	N76D	S103A	V104I	M2228								
N76D	S103A	V104I	Q137R	M222S								
N76D	S103A	V104I	Q109R	M222S	N248S							
N76D	S103A	V1041	M222S	H249R								
V68A	N76D	S103A	V104I	G159D	Q236H	Q245R	N261D					
V68A	N76D	S103A	V1041	S141N	G159D	Q236H	Q245R	T255S				
V68A	N76D	S103A	V104I	G159D	Q236Н	Q245R	R247H					
V68A	N76D	S103A	V104I	G159D	4174V	N204D	Q236H	Q245R				
V68A	N76D	\$103A	V104I	G159D 1	N204D	Q236H	Q245R		T			

/68A	N76D	S103A	V104I	A133V	G159D	N218D	Q236H	Q245R			
/68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R				
/68A	N76D	S103A	V104I	G159D	A194I	V203A	Q236H	Q245R			
)12R	N76D	S103A	V104I	M222S	Q245R						
N76D	S103A	V104I	A232V	Q245R							 L
24T	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N252K				
V68A	N76D	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	T260A		ļ
Q12R	N76D	S103A	1104T	M222S	V244I	Q245R					
Q12R	N76D	S103A	M222S	P210T	Q245R						 ļ
Q12R	N76D	S103A	1104T	S130T	M222S	Q245R					
T22K	V68A	N76D	S103A	V104I							
V68A	N76D	S103A	V104I	N184D							
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	T				
V68A	S103A	V104I	N140D	G159D	A232V		Q245R	N252K			
N43S	V68A	S103A	V104I	G159D	A232V		I Q245R	T			
N438	V68A	S103A	V104I	G159D	A232V		I Q245R				
	V68A	S103A	V104I	G159D	A232V		1 Q245R				
N43D	S87G	S103A		G159D	A232V		1 Q245R		R275S		
V68A	N76D	S103A		S130T	M222S		R N248S				
Q12R	N76D	S103A		S130T	A215V		S Q245R				
Q12R	N76D	S103A		S130T	M222S		A Q245R				
Q12R		S103A		S130T	A215T		S Q245R				
Q12R	N76D N76D	S103A		S130T	M222S		R N261E				
Q12R			S130T	M222S	1						
N76D			·	S130T	N218D		S 0245F	L262S	N269D		
Q12R		N76D			S130T		S Q245				
Q12R				S130T	R170S		D M222				
Q12R				S130T	M2225		R V268				
Q12R					M2225		S Q245				
Q12R							SR L257		1		
V68A									 		
V68A							5H Q245		_	+	
V68/							SR N248			1	
R100							6H Q245		_	+-	
V68/	S103	A V104	I G159	D V2031	A232	v Q23	6H Q245	K			

V68A	S103A	V1041	G159D	4232V	Q236H	K237E	Q245R			 	
V68A	N76D	179N	S103A	V104I	G159D	A232V	Q236H	Q245R			
V68A	S103A	V104I	G159D	N183D	A232V	Q236H	Q245R			 	
V68A	S103A	V1041	G159D	A174V	Q206L	A232V	Q236H	Q245R			
V68A	S103A	V104I	G159D	S188C	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	A230T	A232V	Q236H	Q245R				
V68A	A98T	S103A	V104I	G159D	A232V	Q236H	Q245R			 	
V68A	S103A	V104I	G159D	A215T	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248S			 	
V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R				
V68A	N76D	S103A	V104I	G159D	P210R	A232V	Q236H	Q245R			
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V			
N76D	S103A	V104I	A232V	Q236H	Q245R	L257V					
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	L257V	R275H			
N76D	S103A	V1041	L257V	R275H							
V68A	S103A	V104I	G159D	T224A	A232V	Q236H	Q245R	1.257V			
N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V				<u> </u>
V68A	N76D	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R			
V68A	N76D	S103A	V104I	G159D	G211R	A232V	Q236H	Q245R			
V68A	N76D	S103A	V104I	G159D	G211V	A232V	Q236H	Q245R			
Q12R	V68A	N76D	S103A	V104I	G159D	Y214L	A232V	Q236H	Q245R		
V68A	N76D	S103A	V104I	G159D	A215R	A232V	Q236H	Q245R			
Q12R	V68A	N76D	S103A	V104l	G159D	A232\	Q236H	Q245R			
G20R	V68A	N76D	S103A	V104I	G159D		Q236H		S259G		
V68A	S87R	N76D	S103A	V1041	G159D	A232\	Q236H	Q245R	T260V		<u> </u>
V68A		S103A	V104I	G159D	A232V	Q236I	1 Q245R	N261G			<u> </u>
V68A		S103A	V1041	G159D			1 Q245R				<u> </u>
N76D			A232V			Q2451				<u> </u>	
V68A		S103A	V104I	G159D		A232	Q236H	Q245R		<u> </u>	
Q12R		V68A	N76D	S103A	V104I		D A232V		Q245R		
N76D			A232V								
N76D			G159D			\top	H Q245R				
N76D			V1471	G159D	7		H Q245R		K251R		
Q12R		N76D	S103A		G159I		V Q2361		A272S		
				G159E			L A232V				
V68A		S103A				1			1	1	
V68A	N76D	S103A	V104I	G159E	A232\	10236	H Q245F	13230K		 	

V68A_	N76D	S103A	V104I	G159D	Q206R	A232V	Q236H	Q245R				
K27R	V68A_	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R				
V68A	N76D	S103A	V104I	N116T	G159D	R170S	N185S	A232V	Q236H	Q245R		
G61E	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
N43D	V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212P	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	S99N	G159D	N184D	A232V	Q236H	Q245R	N248D	N252K		
S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K					
V68A	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	Q109R	G159D	A232V	Q236H	Q245R	N248D	N252K			
G20R	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	Y209F	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	N261D			
V68A	S103A	V1041	G159D	N185D	A232V	Q236H	Q245R	N248D	N252K	<u> </u>		
V68A	S103A	V104I	G159D	P210R	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	P210T	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	P210S	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	N185D	P210L	A232V	Q236H	Q245R	N248D	N252K		
V68A	S103A	V104I	G159D	P210L	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212A	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	T213E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	T213S	A232V	Q236H	Q245R	N248D	N252K				
V68A	A103V	V104I	G159D	T213E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K	<u> </u>		
V68A	S103A	V104I	G159D	T213G	A232V	Q236H	Q245R	N248D	N252K	<u> </u>	ļ	
V68A	S103A	V1041	G159D	A215V	A232V	Q236H	Q245R	N248D	N252K	<u> </u>		
V68A	S103A	V104I	G159D	A215R	A232V	Q236H	Q245R	N248D	N252K		<u> </u>	
V68A	S103A	V104I	G159D	S216T	A232V	Q236H	Q245R	N248D	N252K			L
V68A	S103A	V104I	G159D	S216V	A232V	Q236H	Q245R	N248D	N252K	<u> </u>		
V68A	S103A	V104I	G159D	S216C	A232V	Q236H	Q245R	N248D	N252K		<u> </u>	
G20A	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			<u> </u>
V68A	S103A	V104I	G159D	N173D	A232V	Q2361	1 Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245F	N248D	K251V	N252K			
V68A	S103A	V104I	G159D	Q206R	A232V	Q2361	1 Q245R	N248D	N252K			

VERA \$1303A V1041 G159D A232V Q236H Q245R N248D N252L P5955 V68A \$103A V1041 G159D A232V Q236H Q245R N248D N252F V68A \$103A V1041 G159D A232V Q236H Q245R N248D N252K 5256N V68A \$103A V1041 G159D A232V Q236H Q245R N248D N252K 5256N V68A \$103A V1041 G159D A232V Q236H Q245R N248D N252K 5256R V68A \$103A V1041 G159D A232V Q236H Q245R N248D N252K 5256R V68A \$103A V1041 G159D A232V Q236H Q245R N248D N252K 7256R V68A \$103A V1041 G159D A232V Q236H Q245R N248D N252K N260B V68A <													
P355	V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252F				
V98A S103A V1041 G159D A232V Q236H Q245R N248D N252K T255V PART V68A S103A V1041 G159D A232V Q236H Q245R N748D N252K S256R PART V68A S103A V1041 G159D A232V Q236H Q245R N248D N252K S256E PART V68A S103A V1041 G159D A232V Q236H Q245R N248D N252K S256R PART V68A S103A V1041 G159D A232V Q236H Q245R N248D N252K Q258D PART V68A S103A V1041 G159D A232V Q236H Q245R N248D N252K N268D PART V68A S103A V1041 G159D A232V Q236H Q245R N248D N252K N261D PART V68A S103A V1041 G159D A232V Q236H	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252L				
Versit	P55S	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252F			
	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	T255V			
Vora	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	S256N			
V68A \$103A \$V104I G159D A232V \$Q236H \$Q245R \$N248D \$N252K \$T260R V68A \$103A \$V104I G159D A232V \$Q236H \$Q245R \$N248D \$N252K \$L257R \$\$\$\$ V68A \$103A \$V104I G159D A232V \$Q236H \$Q245R \$N248D \$N252K \$N269D 18V \$V68A \$103A \$V104I \$G159D A232V \$Q236H \$Q245R \$N248D \$N252K \$N269D \$V68A \$103A \$V104I \$G159D A232V \$Q236H \$Q245R \$N248D \$N252K \$N261D \$V68A \$103A \$V104I \$G159D A232V \$Q236H \$Q245R \$N248D \$N252K \$N261D \$V68A \$103A \$V104I \$G159D A232V \$Q236H \$Q245R \$N248D \$N252K \$N261D \$V68A \$103A \$V104I \$G159D A232V \$Q236H \$Q245R \$N248D \$N252	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	S256E			
VORBA \$103A \$V1041 \$G159D \$A232V \$Q236H \$Q245R \$N248D \$N252K \$L257R \$\cdots\$ V68A \$103A \$V1041 \$G159D \$A232V \$Q236H \$Q245R \$N248D \$N252K \$Q258D \$\cdots\$ 18V \$V68A \$103A \$V1041 \$Q159D \$A232V \$Q236H \$Q245R \$N248D \$N252K \$N269D \$V68A \$103A \$V1041 \$Q159D \$A232V \$Q236H \$Q245R \$N248D \$N252K \$T260E \$V68A \$103A \$V1041 \$G159D \$A232V \$Q236H \$Q245R \$N248D \$N252K \$N261D \$V68A \$103A \$V1041 \$G159D \$A232V \$Q236H \$Q245R \$N248D \$N252K \$N261D \$V68A \$103A \$V1041 \$G159D \$A232V \$Q236H \$Q245R \$N248D \$N252K \$N261D \$V68A \$103A \$V1041 \$G159D \$A232V \$Q236H \$Q245R <td>V68A</td> <td>S103A</td> <td>V104I</td> <td>G159D</td> <td>A232V</td> <td>Q236H</td> <td>Q245R</td> <td>N248D</td> <td>N252K</td> <td>S256R</td> <td></td> <td></td> <td></td>	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	S256R			
VORBA \$103A \$V1041 G159D A232V \$Q236H \$Q245R	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	T260R			
No.	V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K	L257R			
V68A S103A V104I N116S G159D A232V Q236H Q245R N248D N252K T260E V68A S103A V104I G159D A232V Q236H Q245R N248D N252K N26ID V68A S103A V104I G159D A232V Q236H Q245R N248D N252K N26ID V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K N26ID V68A S103A V104I G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D S101T S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D <	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	G258D			
V68A S103A V104I G159D A232V Q236H Q245R N248D N252K N261R N261A V68A S103A V104I G159D A232V Q236H Q245R N248D N252K N261D V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K N261D V68A S103A V104I G159D A232V Q236H Q245R N248D N252K — S103A V104I G159D A232V Q236R Q245R N248D N252K — N18S V68A S103A V104I G159D A232V Q236H Q245R N248D N252K — V68A S103A V104I G159D A232V Q236H Q245R N248D N252K — V68A N76D S101T S103A V104I G159D A232V Q236H Q245R N248D N252K	18V	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	N269D		
V68A S103A V104I G159D A232V Q236H Q245R N248D N252K N261R V68A S103A V104I G159D A232V Q236H Q245R N248D N252K N261D V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236H Q245R N248D N252K S103A V104I G159D A232V Q236H Q245R N248D N252K	V68A	S103A	V1041	N116S	G159D	A232V	Q236H	Q245R	N248D	N252K	T260E		
V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D		N261R			
V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A V104I G159D A232S Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236R Q245R N248D N252K N18S V68A S103A V104I G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D S101T S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D S80 S103A V104I G159D A232V Q236H Q245R N248D N252K S103A V104I G159D A232V Q236H Q245R N248D N252K S103A V104I S130A	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R		N252K			
V68A S103A V104I G159D A232V Q236R Q245R N248D N252K N18S V68A S103A V104I G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236H Q245V N248D N252K V68A N76D S101T S103A V104I G159D A228V A232V Q236H Q245R N248D N252K T33S V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D S103A V104I G159D P210L T213R A232V Q236H Q245R N248D N252K G61E V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I S130A G159D A23	V68A	S103A	V1041	A232V	Q236H	Q245R	N248D	N252K					
N18S V68A S103A V104I G159D A232V Q236H Q245R N248D N252K	S103A	V104I	G159D	A232S	Q236H	Q245R	N248D	N252K					
V68A S103A V104I G159D A232V Q236H Q245V N248D N252K V68A N76D S101T S103A V104I G159D T213R N218S A232V Q236H Q245R T260A V68A S103A V104I G159D A228V A232V Q236H Q245R N248D N252K T33S V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D E89D S103A V104I G159D P210L T213R A232V Q236H Q245R N248D N252K G61E V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I S130A G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D	V68A	S103A	V104I	G159D	A232V	Q236R	Q245R	N248D	N252K				
V68A N76D S101T S103A V104I G159D T213R N218S A232V Q236H Q245R T260A V68A S103A V104I G159D A228V A232V Q236H Q245R N248D N252K T33S V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D E89D S103A V104I G159D P210L T213R A232V Q236H Q245R T260A G61E V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I S130A G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I A133S Q137R G159D A232V Q236H Q245R N248D N252K G61E S103A V104I G159D <t< td=""><td>N18S</td><td>V68A</td><td>S103A</td><td>V104I</td><td>G159D</td><td>A232V</td><td>Q236H</td><td>Q245R</td><td>N248D</td><td>N252K</td><td></td><td></td><td></td></t<>	N18S	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A S103A V104I G159D A228V A232V Q236H Q245R N248D N252K T33S V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D E89D S103A V104I G159D P210L T213R A232V Q236H Q245R T260A G61E V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K S103A V104I G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I A133S Q137R G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I A133V G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236H Q245R N248D	V68A	S103A	V104I	G159D	A232V	Q236H	Q245V	N248D	N252K				
T33S V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K V68A N76D E89D S103A V104I G159D P210L T213R A232V Q236H Q245R T260A G61E V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K S103A V104I G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I S130A G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I A1338 Q137R G159D A232V Q236H Q245R N248D N252K G61E S103A V104I G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D S160V A232V Q236H Q245R N248D	V68A	N76D	S101T	S103A	V104I	G159D	T213R	N218S	A232V	Q236H	Q245R	T260A	
V68A N76D E89D S103A V104I G159D P210L T213R A232V Q236H Q245R T260A G61E V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K S103A V104I G159D P210I A232V Q236H Q245R N248D N252K G61E V68A S103A V104I S130A G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I A133S Q137R G159D A232V Q236H Q245R N248D N252K G61E S103A V104I G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D S160V A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D S160V A232V Q236H Q245R	V68A	S103A	V104I	G159D	A228V	A232V	Q236H	Q245R	N248D	N252K			
G61E V68A N76D S103A V104I G159D A232V Q236H Q245R N248D N252K S103A V104I G159D V205I P210I A232V Q236H Q245R N248D N252K G61E V68A S103A V104I S130A G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I A133S Q137R G159D A232V Q236H Q245R N248D N252K G61E S103A V104I A133V G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D S160V A232V Q236H Q245R N248D N252K S3L G61E V68A N76D S103A V104I G159D S167F A232V <	T33S	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K		
S103A V104I G159D V205I P210I A232V Q236H Q245R	V68A	N76D	E89D	S103A	V1041	G159D	P210L	T213R	A232V	Q236H	Q245R	T260A	
G61E V68A S103A V104I S130A G159D A232V Q236H Q245R N248D N252K G61E V68A S103A V104I A133S Q137R G159D A232V Q236H Q245R N248D N252K G61E S103A V104I G159D A232V Q236H Q245R N248D N252K	G61E	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K		
G61E V68A S103A V104I A133S Q137R G159D A232V Q236H Q245R N248D N252K G61E S103A V104I A133V G159D A232V Q236H Q245R N248D N252K	S103A	V1041	G159D	V2051	P2101	A232V	Q236H	Q245R			ļ		
G61E S103A V104I A133V G159D A232V Q236H Q245R N248D N252K V68A S103A V104I G159D A232V Q236H Q245R N248G N252K	G61E	V68A	S103A	V104I	S130A	G159D	A232V	Q236H	Q245R	N248D	N252K		
V68A S103A V104I G159D A232V Q236H Q245R N248G N252K V68A S103A V104I G159D N218S A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D S160V A232V Q236H Q245R N248D N252K S3L G61E V68A N76D S103A V104I A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D S167F A232V Q236H Q245R N248D N252K G97E S103A V104I G159D A232V Q236H Q245R N248D N252K	G61E	V68A	S103A	V1041	A1338	Q137R	G159D	A232V	Q236H	Q245R	N248D	N252K	<u> </u>
V68A S103A V104I G159D N218S A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D S160V A232V Q236H Q245R N248D N252K S3L G61E V68A N76D S103A V104I A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D S167F A232V Q236H Q245R N248D N252K G97E S103A V104I G159D A232V Q236H Q245R N248D N252K	G61E	S103A	V1041	A133V	G159D	A232V	Q236H	Q245R	N248D	N252K			<u> </u>
G61E V68A S103A V104I G159D S160V A232V Q236H Q245R N248D N252K S3L G61E V68A N76D S103A V104I A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D S167F A232V Q236H Q245R N248D N252K G97E S103A V104I G159D A232V Q236H Q245R N248D N252K	V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248G	N252K				<u> </u>
S3L G61E V68A N76D S103A V104I A232V Q236H Q245R N248D N252K G61E V68A S103A V104I G159D S167F A232V Q236H Q245R N248D N252K G97E S103A V104I G159D A232V Q236H Q245R N248D N252K	V68A	S103A	V104I	G159D	N218S	A232V	Q236H	Q245R	N248D	N252K			
G61E V68A S103A V104I G159D S167F A232V Q236H Q245R N248D N252K G97E S103A V104I G159D A232V Q236H Q245R N248D N252K	G61E	V68A	S103A	V104I	G159D	S160V	A232V	Q236H	Q245R	N248D	N252K		<u> </u>
G97E S103A V104I G159D A232V Q236H Q245R N248D N252K	S3L	G61E	V68A	N76D	S103A	V104I	A232V	Q236H	Q245R	N248D	N252K		
	G61E	V68A	S103A	V104I	G159D	S167F	A232V	Q236H	Q245R	N248D	N252K		<u> </u>
	G97E	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K				
A98D S103A V1041 G159D A232V Q236H Q245R N248D N252K	A98D	S103A	V1041		A232V		Q245R	N248D	N252K				

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S99E	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101E	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S191G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
G102A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V1041	S106E	G159D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	Q109E	G159D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	N261R			
S103A	V104I	Q109R	G159D	A232V	Q236H	Q245R	N248D	N252K			
N62D	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	G159D	N184D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	G159D	S166D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	G159D	L217E	A232V	Q236H	Q245R	N248D	N252K			
G20R	N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K	
N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S103A	V104I	G159D	Q206R	L217E	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V104I	G159D	Q206R	A232V	Q236H	Q245R	N248D	N252K		
S103A	V104I	S130G	G159D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V1041	P131V	G159D	A232V	Q236H	Q245R	N248D	N252K			
K27N	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
T38G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
T38A	N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A		
V68A	N76D	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	T260A	E271G	
V68A	N76D	S103A	V104I	G159D	Y209W	T213R	A232V	Q236H	Q245R	T260A	
V68A	N76D	S103A	V104I	G159D	P210I	T213R	A232V	Q236H	Q245R	T260A	
V68A	N76D	S103A	V104I	G159D	V205I	T213R	A232V	Q236H	Q245R	T260A	
V68A	N76D	S103A	V104I	G159D	P2101	A232V	Q236H	Q245R	T260A		
V68A	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A			
N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A			
V68A	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	_			
V68A	S103A	V1041	G159D	P2101	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	A230V	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	L126F	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	V2051	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	P210L	A232V	Q236H	Q245R				
S103A	V104I	G159D	A230V	Q236H	Q245R						
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	T260A				

											
S103A	V1041	G159D	A232V	Q236H	Q245R				<u> </u>		
V68A	S103A	V104I	G159D	A174V	A232V	Q236H	Q245R	1.257V			
V68A	S103A	V104I	G159D	A194S	A232V	Q236H	Q245R	L257V			
V68A	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	L257V			
S103A	V104I	G159D	A232V	Q236H	Q245R	L257V					
V68A	N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A	N261	
										w	
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	1.257V	N261W			
S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A				
S103A	V104I	G159D	P210I	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	L257V				
V68A	N76D	S103A	V104I	G159D	P210L	T213R	A232V	Q236H	Q245R	T260A	
Q12R	S103A	V104I	G159D	Y209W	T213R	A232V	Q236H	Q245R	T260A		
S103A	V104I	Y209W	A232V	Q236H	Q245R	L257V					
S103A	V104I	G159D	V2051	P210I	T213R	A232V	Q236H	Q245R	T260A		
S103A	V104I	G159D	V205I	Y209W	A232V	Q236H	Q245R	T260A			
V68A	S103A	V104I	G159D	V2051	Y209W	P210I	A232V	Q236H	Q245R		
S103A	V104I	G159D	V2051	Y209W	P210I	A232V	Q236H	Q245R	L257V		
S103A	V104I	G159D	V2051	Y209W	A232V	Q236H	Q245R	1.257V			
V68A	S103A	V104I	G159D	V2051	Y209W	P210I	A232V	Q236H	Q245R	T260A	
S103A	V104I	G159D	V2051	Y209W	P210I	A232V	Q236H	Q245R			
S103A	V104I	G159D	Y209W	P210I	A232V	Q236H	Q245R				
S103A	V104I	G159D	V2051	P210I	A232V	Q236H	Q245R				
V68A	S103A	V104I	S128L	G159D	A232V	Q236H	Q245R				
A48V	S103A	V104I	G159D	A230V	Q236H	Q245R					
A48V	V68A	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R			
A48V	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K		
A48V	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V	N261W		
G102A	S103A	V1041	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K		
Q12R	G102A	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R		N252K	
S101G	G102A	S103A	V104I		\$212G	A232V		Q245R	N248D	N252K	
A98L	G102A	S103A	V104I		S212G	A232V			N248D	N252K	
G102A	S103A	V104I	G159D	T213R		Q236H		N248D	N252K		
S103A	V104I					Q245R		N252K			
S103A						Q245R		N252K			
						Q245R		N252K	- 		
		/_		· •	Assou	Z*47K	עסרגרו	IATOTA		لــــــل	

S103A	V104I	G159D	A232V	Q236H	V244T	Q245R	N248D	N252K				
S103A	V1041	G159D	A232V	Q236H	V244A	Q245R	N248D	N252K				
N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K	S256R		
Q12R	N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S101G	S103A	V104I	G159D	N185D	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	G159D	Q206E	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	G159D	T213Q	A232V	Q236H	Q245R	N248D	N252K			
A98L	G102A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G	G102A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
A98L	G102A	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K		
A98L	G102A	S103A	V104I	G159D	S212G	A232V	Q236H	N248D	N252K			
N62D	S103A	V104I	Q109R	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V104I	G159D	S212G	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S101G	S103A	V104I	G159D	S212G	T213R	A232V	Q236H	Q245R	N248D	N252K	
S103A	V104I	G159D	A232V	Q245R	N248D	N252K						
S103A	V104I	G159D	A230V	Q245R								
N62D	S103A	V104I	S130G	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S101G	S103A	V104I	S130G	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V1041	S128G	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	S128L	G159D	A232V	Q236H	Q245R	N248D	N252K			
N62D	S101G	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V1041	S128G	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V1041	S128L	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		ļ
S101G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	T260A	<u> </u>		
S101G	S103A	V104I	P131V	G159D	A232V	Q236H	Q245R	N248D	N252K	ļ		
A98V	S101G	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K	ļ	<u> </u>	
S99G	S101G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K	ļ		
			ļ		<u> </u>			<u> </u>	<u> </u>	ļ	ļ	
S101G	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	N248D	N252K	ļ		
S101G	S103A	V104I	G159D	P2101	A232V	Q236H	Q245R	N248D	N252K	<u> </u>		
S101G	S103A	V104I	G159D	V2051	A232V	Q236H	Q245R	N248D	N252K	ļ	ļ	
S101G	S103A	V104I	G159D	A230V	Q236H	Q245R				1	ļ	ļ
S101G	S103A	V104I	G159D	A194P	A232V	Q236H	Q245R	N248D	N252K			<u> </u>
N76D	\$101G	S103A	V104I	G159D	A194P	A232V	Q236H	Q245R	N248D	N252K		
S101G	S103A	V104I	G159D	A230V	A232V	Q236H	Q245R	N248D	N252K		<u> </u>	<u></u>

N62D S103A V104I	G159D N185D Q206E	T213R A232V Q236H	Q245R N248D N252K E271Q

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table I except for the following substitution sets of Table III:

Table III

						duic III				
76	103	104	259							
76	86	103	104							
76	103	104	130							
76	99	103	104	204						
76	103	104	242							
76	103	104	104	182	198					
21	76	103	104	182						
76	103	104	119	137						
76	103	104	173	222						
61	76	103	104	222						
68	76	103	104	116	159	170	185	232	236	245

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table IV:

Table IV

76	103	104	222	245							
76	103	104	222	249							
68	103	104	159	232	236	245	252				
68	76	103	104	159	213	232	236	245	260		
22	68	76	103	104							
68	103	104	159	232	236	245	248	252			
68	103	104	159	232	236	245					
68	103	104	140	159	232	236	245	252		-	
43	68	103	104	159	232	236	245	252			
43	68	103	104	159	232	236	245				
12	76	103	104	130	222	245	261				

									37									1
76	103	104	130	2:	22	245		\Box										
68	103	104	159	2	32	236	24	45	257						\perp			
68	76	103	104	1	59	210	2	32	236	5	245				_			-
68	103	104	159) 2	24	232	2	36	24:	5	257	ļ			_}			}
76	103	104	159	9 2	32	236	2	45	25	7								4
68	76	103	10	4 1	59	211	2	32	23		245							-
12	68	76	10	3 1	104	159	\bot	14	23		236	1	245			-		4
68	76	103	10	4	159	215	┷	232	23		245	<u> </u>				_		\dashv
12	68	76	10	3	104	159		232	23		245	↓_	250			_		4
20	68	76	10)3	104	159		232	23		245		259			-		4
68	76	87	10)3	104	15	9	232		36	245	_	260			<u> </u>		4
68	76	10	3 10)4	159	23	2	236	<u> </u>	45	261	1				-		4
12	48	68	3 7	6	103	10		159		32	236	_	245			\vdash		\dashv
76	103	10	4 1	59	192	23		236		45		1	251	ļ		┼-		\dashv
76	103	10	4 1	47	159	-		236		45	248	\bot	251	-		+		\dashv
12	68	7	6 1	03	104	╄-	-	232		36	245	+	272	├		+		\dashv
68	76	10	3 1	04	159		33	206		32	236	_	245	┼		+		
68	3 76	10)3 1	04	159		32	236		245	256	4		┼		+		
6	8 76			04	159		06	232		236	245	+		+-		+		
2	7 68			103	104		59	232		236	245 248	-	252	+-		+		
6				159	212	_	32	236	Ц_	245	240	-		+		+		
L_)3 10			232	23	_	45	248	4	252	248	\dashv	252	+		+		
6	8 10			159	20		32	236	-	245	248		252	+		+		
6	8 10			109	↓	_	232	236		245	248		252	+		\dashv		
<u> </u>			03	104	1-	_	232	236	_	245 245	248	_	252	+		\dashv		
_ L			104	159	4_		232	230	-	245	248		252			-		
			104	159	—	_+	232	ļ		245			252			-		
\perp			104	159	ᆜ—	1	232			245			252					_
<u> </u>	-		104	159	-		232			243				+				
L			104	213	-	32	236		_	248			252	+			-	
			104	159		15	232	—		245			252				-	
	68	103	104	15	2	16	23:	2 23	0,0	243							<u> </u>	

68	103	104	159	232	236	245	248	252		
103	104	159	232	236	245	248	252	255		
		159	232	236	245	248	252	256		
		159	232	236	245	248	252	260		
		159	228	232	236	245	248	252		
	ļ		104	159	210	213	232	236	245	260
	<u> </u>	<u> </u>	<u> </u>	232	236	245	248	252		
	103 103 103 103 76 103	103 104 103 104 103 104 103 104 76 89	103 104 159 103 104 159 103 104 159 103 104 159 76 89 103	103 104 159 232 103 104 159 232 103 104 159 232 103 104 159 228 76 89 103 104	103 104 159 232 236 103 104 159 232 236 103 104 159 232 236 103 104 159 232 236 103 104 159 228 232 76 89 103 104 159	103 104 159 232 236 245 103 104 159 232 236 245 103 104 159 232 236 245 103 104 159 232 236 245 103 104 159 228 232 236 76 89 103 104 159 210	103 104 159 232 236 245 248 103 104 159 232 236 245 248 103 104 159 232 236 245 248 103 104 159 232 236 245 248 103 104 159 228 232 236 245 76 89 103 104 159 210 213	103 104 159 232 236 245 248 252 103 104 159 232 236 245 248 252 103 104 159 232 236 245 248 252 103 104 159 228 232 236 245 248 76 89 103 104 159 210 213 232 76 89 103 104 159 210 213 232	68 103 104 139 232 236 245 248 252 255 103 104 159 232 236 245 248 252 256 103 104 159 232 236 245 248 252 260 103 104 159 228 232 236 245 248 252 260 103 104 159 228 232 236 245 248 252 76 89 103 104 159 210 213 232 236 76 89 103 104 159 210 213 232 236	68 103 104 139 232 236 245 248 252 255 103 104 159 232 236 245 248 252 256 103 104 159 232 236 245 248 252 260 103 104 159 228 232 236 245 248 252 76 89 103 104 159 210 213 232 236 245 76 89 103 104 159 210 213 232 236 245

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table V:

Table V

					-	aute v					
68A	S103A	V104I	G159D	A228V	A232V	Q236H			N252K		
		V1041	G159D	N218S	A232V	~~~	~ =		N252K		
320R		S103A	V104I	G159D	A232V	Q236H	Q245R		N252K		
/68A	N76D	E89D	S103A	V104I	G159D	P210L			Q236H	2245R	1260A
/68A	S103A	V104I	G159D	A232V	Q236H	Q245R			S256R		ļ
/68A	S103A	V104I	G159D	A232V	Q236H	Q245R			T260R		
768A	S103A	V104I	G159D	A232V	Q236H	Q245R	1	N252K	T255V		
V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D		S256N		
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R		N252L			├
V68A	S103A	V104I	G159D	T213R	A232V	Q236H		N248D	N252K		ļ
V68A	S103A	V1041	G159D	A215V	A232V	Q236H	1	N248D	N252K		
V68A	S103A	V104I	G159D	A215R	A232V	Q236H		N248D	N252K		↓
V68A	S103A	V1041	G159D	S216T	A232V	Q236H	1 -	N248D	N252K		↓
V68A	S103A	V1041	G159D	S216V	A232V	Q236H	1	N248D	N252K		+
V68A	S103A	V1041	T213S	A232V	Q236H	Q245R	N248D	1	1	<u> </u>	+
V68A	S103A	V104I	G159D	P210L	A232V	Q236H	1 '	N248D			-
V68A	S103A	V104I	G159D	S212C	A232V	Q236H	1	N248D			-
V68A	S103A	V1041	G159D	S212G	A232V	Q236H		N248D	N252K	\	
S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K		1	 	-
V68A	S103A	V1041	G159D	Y209W	A232V	Q236H	1	N248D			
V68A	S103A	V1041	Q109R	G159D	A232V	Q236H	1	N248D			
G20R	V68A	S103A	V104I	G159D	A232V	Q236H		N248E			
V68A	S103A	V104I	G159D	Y209F	A232V	Q236H			N2521	4-	
Q12R	N76D	S103A	I104T	S130T	M222S	Q245R	N2611	1		-	
N76D		1104T	S130T	M222S	Q245R					-	
N76D		V1041	M222S	H249R							
N76D		V104I	M222S	Q245R						-	
N76D		4 V1041	G159D	Y192F	A232V	Q2361	1	1		4-	
N76D			V1471	G159E	A232V	Q2361	H Q245	R N248	S K251	K	L_

					37						
12R	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R	A272S		
	1	S103A	V104I	G159D	N183K	Q206L	A232V	Q236H	Q245R		
768A		\$103A	V104I	G159D	A232V	Q236H	Q245R	S256R			
/68A		S103A	V104I	G159D	Q206R	A232V	Q236H	Q245R			
27R		N76D	S103A	V104I	G159D	A232V	Q236H	Q245R			
	A48V	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R		
012R V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N261W			\top
	N76D	S103.4	V104I	G159D	G211R	A232V	Q236H	Q245R			
V68A	N76D	S103A	V1041	G159D	G211V	A232V	Q236H	Q245R			
V68A		N76D	S103A	V104I	G159D	Y214L	A232V	Q236H	Q245R		
Q12R	V68A	S103A	V1041	G159D	A215R	A232V	Q236H	Q245R			\top
V68A	N76D	N76D	S103A	V1041	G159D	A232V		Q245R	†		1
Q12R	V68A	N76D	S103A	V104I	G159D	A232V		Q245R	S259G		1
G20R	V68A	S87R	S103A	V104I	G159D	A232V	Q236H	Q245R	T260V		
V68A	N76D	V104I	G159D	A232V	Q236H	Q245R	1.257V	1	T		1
N76D	S103A	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A		
V68A	N76D	N76D	S103A	V1041	-		+	1	 	\vdash	1
T22K	V68A	1	V104I	G159D	P210R	A232V	Q236H	Q245R	+	1	+
V68A	N76D	S103A		S212P	A232V	Q236H	1 '	N248D		:-	+-
V68A	S103A	V104I	G159D	T224A	A232V	O236H		L257V		+-	1
V68A	S103A		G159D	A232V	Q236H	Q245R			+	+	+-
V68A	S103A		G159D			Q245R				+-	+-
V68A	S103A		G159D			Q245R			-	+	+
V68A	S103A		G159D					+	+	+	+
V68A	S103A		G159D					R N2521	<u> </u>	+-	\top
V68A			N140D	G159D			1	R N2521		+-	\top
N43S	V68A	S103A		G159D					+-	+	十
N43K				G159D			1	R N252	K -	+-	
N43D									+-	+-	\dashv
V68A	S103/	V1041	G159I	AZSZV	Q236H	(42,43					

A highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12/102/103/104/159/212/232/236/245/248/252; 12/76/103/104/130/170/185/222/243/245; 12/76/103/104/130/222/245; 12/76/103/104/130/222/245/261; 12/76/103/104/222/245; 62/103/104/159/213/232/236/245/248/252; 61/68/103/104/159/232/236/245/248/252; $62/103/104/109/159/213/232/236/245/248/252; \ 62/103/104/159/232/236/245/248/252; \\$ 62/101/103/104/159/212/213/232/236/245/248/252; 62/103/104/130/159/213/232/236/245/248/252; 68/103/104/159/232/236/245/248/252/270; 68/103/104/159/210/232/236/245/248/252; 68/103/104/159/185/232/236/245/248/252; 68/76/103/104/159/209/232/236/245; 68/103/104/159/230/232/236/245; 68/103/104/213/232/236/245/248/252; 68/103/104/232/236/245/248/257/275; 68/103/104/159/209/232/236/245; 68/103/104/159/232/236/245/248/252; 68/76/103/104/159/236/245; 68/76/103/104/159/236; 68/103/104/159/232/236/245/252; 68/76/103/104/159/232/236/245; 68/103/104/159/232/236/245/257; 68/103/104/159/232/236/245; 68/76/103/104/159/215/232/236/245; 68/76/103/104/159/211/232/236/245; 68/103/104/159/213/232/236/245/260; 68/103/104/159/210/232/236/245; 68/103/104/159/236; 68/76/103/104/159/213/232/236/245/260; 68/103/104/159/236/245; 68/76/103/104/159/210/232/236/245/260; 68/76/103/104/159/236/245; 68/103/104/159/183/232/236/245/248/252; 68/103/104/159/213/232/236/245; 68/103/104/232/236/245/257/275; 76/103/104/222/245; 76/103/222/245; 76/103/104/159/232/236/245; 76/103/104/159; 76/103/104/159/213/232/236/245/260; 97/103/104/159/232/236/245/248/252; 76/103/104/131/159/232/236/245/248/252; $98/102/103/104/159/212/232/236/245/248/252; \ 98/103/104/159/232/236/245/248/252;$ 102/103/104/159/232/236/245/248/252; 101/103/104/159/232/236/245/248/252; 103/104/159/232/236/245/248/252; 103/104/159/232/236/245; 103/104/159/232/245/248/252; 103/104/159/205/209/232/236/245/257 103/104/159/213/232/236/245/248/252; 103/104/159/205/209/210/232/236/245/257; 103/104/130/159/232/236/245/248/252; 103/104/159/217/232/236/245/248/252; 103/104/159/236/245; 103/104/159/230/236/245; 103/104/131/159/232/236/245/248/252; 103/104/159/248/252/270; 103/104/159/232/236/245/257. 103/104/159/205/209/232/236/245; and

A more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12R/76D/103A/104T/130T/222S/245R; 12R/76D/103A/104I/222S/245R; 12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 12R/76D/103A/104T/130G/222S/245R/261D; 12R/76D/103A/104T/130G/170S/185D/222S/243D/245R; 61E/68A/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K; 62D/103A/104I/159D/213R/232V/236H/245R/248D/252K; 62D/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K; 62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K; 68A/103A/104I/159D/232V/236H/245R/248D/252K/270A; 68A/76D/103A/104I/159D/213R/232V/236H/245R/260A; 68A/103A/104I/159D/236H; 68A/103A/104I/159D/236H/245R; 68A/76D/103A/104I/159D/210I/232V/236H/245R/260A; 68A/103A/104I/159D/183D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/209W/232V/236H/245R; 68A/76D/103A/104I/159D/211R/232V/236H/245R; 68A/76D/103A/104I/159D/215R/232V/236H/245R; 68A/103A/104I/159D/213R/232V/236H/245R/260A; 68A/76D/103A/104I/159D/236H; 68A/76D/103A/104I/159D/236H/245R; 68A/76D/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/252K; 68A/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/257V; 68A/103A/104I/159D/185D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K;

> 68A/103A/104I/159D/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/230V/232V/236H/245R;

68A/76D/103A/104I/159D/209W/232V/236H/245R; 68A/103A/104I/232V/236H/245R/248D/257V/275H; 68A/103A/104I/232V/236H/245R/257V/275H; 68A/103A/104I/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210I/232V/236H/245R; 68A/103A/104I/159D/210L/232V/236H/245R; 68A/103A/104I/159D/213G/232V/236H/245R; 76D/103A/222S/245R; 76D/103A/104I/222S/245R; 76D/103A/104I/159D/232V/236H/245R; 76D/103A/104I/159D; 76D/103A/104I/131V/159D/232V/236H/245R/248D/252K; 76D/103A/104I/159D/213R/232V/236H/245R/260A: 97E/103A/104I/159D/232V/236H/245R/248D/252K; 98L/103A/104I/159D/232V/236H/245R/248D/252K; 98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 101G/103A/104I/159D/232V/236H/245R/248D/252K; 102A/103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/213R/232V/236H/245R/248D/252K; 103A/104I/130G/159D/232V/236H/245R/248D/252K; 103A/104I/159D/230V/236H/245R; 103A/104I/159D/217E/232V/236H/245R/248D/252K; 103A/104I/159D/236H/245R; 103A/104I/159D/248D/252K/270V; 103A/104I/159D/232V/236H/245R; 103A/104I/159D/205I/209W/232V/236H/245R; 103A/104I/159D/232V/236H/245R/257V; 103A/104I/159D/205I/209W/232V/236H/245R/257V; 103A/104I/131V/159D/232V/236H/245R/248D/252K; 103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and 103A/104I/159D/232V/245R/248D/252K.

An even more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12/76/103/104/130/222/245/261; 62/103/104/159/232/236/245/248/252; 62/103/104/159/213/232/236/245/248/252; 62/101/103/104/159/212/213/232/236/245/248/252; 68/103/104/159/232/236/245; 68/103/104/159/230/232/236/245; 68/103/104/159/209/232/236/245; 68/103/104/159/232/236/245/257; 68/76/103/104/159/213/232/236/245/260; 68/103/104/159/213/232/236/245/248/252; 68/103/104/159/183/232/236/245/248/252; 68/103/104/159/185/232/236/245/248/252; 68/103/104/159/185/210/232/236/245/248/252; 68/103/104/159/210/232/236/245/248/252; 68/103/104/159/213/232/236/245; 98/103/104/159/232/236/245/248/252; 98/102/103/104/159/212/232/236/245/248/252; 101/103/104/159/232/236/245/248/252; 102/103/104/159/232/236/245/248/252; 103/104/159/230/236/245; 103/104/159/232/236/245/248/252; 103/104/159/217/232/236/245/248/252; 103/104/130/159/232/236/245/248/252; 103/104/131/159/232/236/245/248/252; 103/104/159/213/232/236/245/248/252; and 103/104/159/232/236/245.

The most highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

> 12R/76D/103A/104T/130T/222S/245R/261D; 62D/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/159D/213R/232V/236H/245R/248D/252K; 68A/103A/104I/159D/209W/232V/236H/245R; 68A/76D/103A/104I/159D/213R/232V/236H/245R/260A; 68A/103A/104I/159D/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/230V/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/257V; 68A/103A/104I/159D/213G/232V/236H/245R/248D/252K; 68A/103A/104I/159D/185D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/213G/232V/236H/245R; 98L/103A/104I/159D/232V/236H/245R/248D/252K; 98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 101G/103A/104I/159D/232V/236H/245R/248D/252K; 102A/103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/230V/236H/245R; 103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/217E/232V/236H/245R/248D/252K; 103A/104I/130G/159D/232V/236H/245R/248D/252K; 103A/104I/131V/159D/232V/236H/245R/248D/252K; 103A/104I/159D/213R/232V/236H/245R/248D/252K; and 103A/104I/159D/232V/236H/245R.

In another preferred embodiment, the protease variants which are the protease enzymes useful in the cleaning compositions of the present invention comprise protease variants including a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin.

While any combination of the above listed amino acid substitutions may be employed, the preferred protease variant enzymes useful for the present invention comprise the substitution, deletion or insertion of amino acid residues in the following combinations:

- (1) a protease variant including substitutions of the amino acid residues at position 62 and at one or more of the following positions 103, 104, 109, 159, 213, 232, 236, 245, 248 and 252;
- (2) a protease variant including substitutions of the amino acid residues at position 212 and at one or more of the following positions 12, 98, 102, 103, 104, 159, 232, 236, 245, 248 and 252;
- (3) a protease variant including substitutions of the amino acid residues at position 230 and at one or more of the following positions 68, 103, 104, 159, 232, 236 and 245;

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- (4) a protease variant including substitutions of the amino acid residues at position 232 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (5) a protease variant including substitutions of the amino acid residues at position 232 and at one or more of the following positions 103, 104, 236 and 245;
- (6) a protease variant including substitutions of the amino acid residues at position 232 and 103 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (7) a protease variant including substitutions of the amino acid residues at position 232 and 104 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (8) a protease variant including substitutions of the amino acid residues at position 232 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (9) a protease variant including substitutions of the amino acid residues at position 232 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (10) a protease variant including substitutions of the amino acid residues at position 232, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- (11) a protease variant including substitutions of the amino acid residues at position 252 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (12) a protease variant including substitutions of the amino acid residues at position 252 and at one or more of the following positions 103, 104, 236 and 245;
- (13) a protease variant including substitutions of the amino acid residues at positions 252 and 103 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (14) a protease variant including substitutions of the amino acid residues at positions 252 and 104 and at one or more of the following positions 12, 61, 62, 68, 97, 98,

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101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;

- (15) a protease variant including substitutions of the amino acid residues at positions 252 and 236 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (16) a protease variant including substitutions of the amino acid residues at positions 252 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- (17) a protease variant including substitutions of the amino acid residues at positions 252, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270; and
- (18) a protease variant including substitutions of the amino acid residues at position 257 and at one or more of the following positions 68, 103, 104, 205, 209, 210, 232, 236, 245 and 275.

A more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table VI) selected from the group consisting of:

Table VI

							able v	<u>. </u>	 		
76	103	104	212	271					 ,		
76	103	104	252	261					 		
76	103	104	212	258					 		
4	76	103	104	159	217	252			 		
12	62	76	103	104	159					<u> </u>	
76	103	104	212	268	271					<u> </u>	ļ
76	87	103	104	212	271						
76	103	104	212	245	271				 		
76	103	104	134	141	212	271				<u> </u>	
76	103	104	212	236	243	271					
20	62	76	103	104					 		
68	76	103	104	159	232	236	245				
76	103	104	232	245							
24	68	76	103	104	159	232	236	245			<u> </u>

	102	104	150	232	236	245	252				T		
68	103	104	159	159		245	252	245	260		-		
68	76	103	104		213	232	236		200				
68	103	104	159	232	236	245	248	252			 		
68	103	104	159	232	236	245		252			┼		
68	103	104	140	159	232	236	245	252_			-		
43	68	103	104	159	232	236	245	252				+	
43	68	103	104	159	232	236	245						
43	68	103	104	159	232	236	245	252					
68	87	103	104	159	232	236	245	252	275				
68	103	104	159	232	236	245	257				-		
68	103	104	116	159	232	236	245		-		-	_	
68	103	104	159	232	236	245	248			 		\dashv	
10	68	103	104	159	232	236	245			<u> </u>	-	_	
68	103	104	159	203	232	236	245				-		
68	103	104	159	232	236	237	245						
68	76	79	103	104	159	232	236	245	ļ			_	
68	103	104	159	183	232	236	245	ļ		<u> </u>			
68	103	104	159	174	206	232	236	245	ļ				
68	103	104	159	188	232	236	245			ļ			
68	103	104	159	230	232	236	245		ļ				
68	98	103	104	159	232	236	245			ļ			
68	103	104	159	215	232	236	245			<u> </u>			
68	103	104	159	232	236	245	248						
68	76	103	104	159	232	236	245	<u> </u>					
68	76	103	104	159	210	232	236	245	<u> </u>				
68	76	103	104	159	232	236	245	257		<u> </u>		\perp	
76	103	104	232	236	245	257					\perp		
68	103	104	159	232	236	245	257	275					
76	103	104	257	275									
68		104	159	224	232	236	245	257					
76		7	\top	232	236	245	257						
68			1				236	245	:				
68			\dashv										
12	_						+						
68		\neg			\neg				\dashv				
12	\neg	_				-				1			

20	(0)	76	102	104	150	232	226	245	250			
20	68	76	103	104	159	+	236	245	259			-
68	87	76	103	104	159	232	236	245	260			
68	76	103	104	159	232	236	245	261				
76	103	104	232	236	242	245						
68	76	103	104	159	210	232	236	245			<u> </u>	
12	48	68	76	103	104	159	232	236	245			
76	103	104	232	236	245							
76	103	₋ 104	159	192	232	236	245				ļ <u>.</u>	
76	103	104	147	159	232	236	245	248	251			
12	68	76	103	104	159	232	236	245	272			
68	76	103	104	159	183	206	232	236	245			<u> </u>
68	76	103	104	159	232	236	245	256				
68	76	103	104	159	206	232	236	245				
27	68	76	103	104	159	232	236	245				
68	76	103	104	116	159	170	185	232	236	245		
61	68	103	104	159	232	236	245	248	252			
43	68	103	104	159	232	236	245	248	252			
68	103	104	159	212	232	236	245	248	252			
68	103	104	99	159	184	232	236	245	248	252		
103	104	159	232	236	245	248	252					
68	103	104	159	209	232	236	245	248	252			
68	103	—	109	159	232	236	245	248	252			
20	68	103	104	159	232	236	245	248	252			
68	103	1 -	159	209	232	236	245	248	252			
68	103	1	159	232	236	245	248	252	261			
68	103		+	185	232	236	245	248	252			
68	103		1	210	232	236	245	248	252			
68	103			185	210	232	236	245	248	252		
68	7	\neg		212	232	236	245	248	252			
68			_	+	232	236	245	248				
68		-	+	 	236	245	248	+		1		
68		+		 	232	236	245				1	1
				 	232	236	245				1	
68				 							+	
20	_					+	245				+-	+
68	\dashv						1		-		-	
68	10:	3 104	1 159	232	236	245	248	251	252	_l		

									_		47 				T				1		Т		\neg	
68	1	03	104	1 1	159	20	6	232	1	236	1:	245	1	248	+	252	+		┼		+-		ㅓ	
68	1	03	104	4	159	23	2	236	\perp	245	1:	248	1	252	+				┼-		╀		ㅓ	
55	T	68	10	3	104	15	9	232	\perp	236	1	<u> 245</u>	4	248	+	252	_		+		╁		ㅓ	
68	1	103	10	4	159	23	32	236	\perp	245	1	248	\bot	252	+	255			+-		+		ᅴ	
68	1	103	10	4	159	23	32	236		245	\perp	248	4	252	+	256	\dashv		+		+			
68	1	103	10)4	159	2:	32	236	5	245	1	248	4	252	<u> </u>	26	\neg		+		+			
68		103	10)4	159	2	32	230	5	245	<u>;</u>	248	3	252	2	25	$\neg \vdash$		+		+			
68		103	10	04	159	2	32	23	6	245	5	24	8	252	\neg	25			+		+			1
8	1	68	10	03	104	1	59	23	2	23	6	24	5	24	_	25		269	_		\dashv			1
68	3	103	1	04	116	1	59	23	2	23	6	24	5	24		25	-	260	+		\dashv			1
68	8	103	1	04	159		232	23	6	24	5	24	8	25		20		<u>. </u>	-+		-			1
6	8	103	1	04	159	<u>)</u>	232	23	6	24	5	24		25		+	61		\dashv					+
6	8	76	1	03	104	1	159	2:	32	23	6	24		24	18	2	52							┧
6	8	103	1	104	23:	2	236	2	45	24	18	 	52_	-		┼-			-					1
1	03	104		159	23	2	236	2	45	+	48_	+	52	-		+						-		-
	68	103	3	104	15	9	232	┪	36	+	45	+	48	+	52	+	 252	-	\dashv			T		_
	18	68	1	103	10	4	159		32	+	36	┪	45	+-	48	+-	232	\vdash				T		
	68	10	3	104	1:	9	232	-	236	+-	45	+-	48	+-	252 232		236	1 2	45	2	60	T		_
	68	76	5	101	10)3	104	-+-	159	+-	213	+-	218	+	248	_	<u>250</u> 252	+-		-		+		_
	68	10	3	104	1.	59	228	_	232	+	236	+	245	十	240 245	-	248	1 2	252	T		1		
	33	6	8	76		03	104	_	159	_	232	+	236 213	+-	232	\dashv	236	+	245	1:	260	T		
	68	7	6	89	_	03	104	_	159	-+-	210	十	213 236	+	24:		248	+-	252			7		
-	61	6	8	76	-	03	10		159	-	232 236	+	245	_		+		T		1		1		
-	103	3 1	04	15		205	21		232	-	232	_	236	_	24	5	248		252	T				
-	61		68	10	-	104	13		159	_	232 159	-	232	_	23		245	+	248		252			
-	61	+	68	10	_	104	13		23	$\neg \uparrow$	236	_	24	_	_ 24	$\neg \uparrow$	252	\neg						
	6	-	103	10		133	+	59	23	$\neg \uparrow$	24	_	24	\neg		52		1						_
}	6	_	103	+		159	_	32	23 23		23		24			48	25	2						
	6	_	103	+	04	159	+	18 59		50	_ <u></u> 23	\neg	23	_		 45	24	8	252	2				
	6	$\neg \uparrow$	68	+-	03	104	1	03		04		32		36		45	24	8	25	2			<u>_</u>	_
	-	3	61	\dashv	58	76	+-	59	_	67		32		36	_	45	24	18	25	2			_	
	\vdash	51	68	+-	03	104		232		36	_	45	 	48	┼─	252							_	
	-	97	103	+	04	159	_	<u>232</u> 232	_	36	\vdash	45	 	48	+-	 252								_
	-	98	103		104		-†-	232 232	+-	236	一	45	+	48	1	252							\perp	_
	\vdash	99	103	+	104	15	-	232 232	+-	236	 	245	+	248		252							1	
		101	10	3	104	13	7	232	1															

												1
102	103	104	159	232	236	245	248	252				
103	104	106	159	232	236	245	248	252				ļ
103	104	109	159	232	236	245	248	252				<u> </u>
103	104	159	232	236	245	248	252	261				<u> </u>
62	103	104	159	232	236	245	248	252				ļ
103	104	159	184	232	236	245	248	252				<u> </u>
103	104	159	166	232	236	245	248	252				ļ
103	104	159	217	232	236	245	248	252				
20	62	103	104	159	213	232	236	245	248	252		
62	103	104	159	213	232	236	245	248	252			
103	104	159	206	217	232	236	245	248	252	ļ		
62	103	104	159	206	232	236	245	248	252			
103	104	130	159	232	236	245	248	252	<u> </u>	ļ	ļ	
103	104	131	159	232	236	245	248	252	 	 		
27	103	104	159	232	236	245	248	252	<u> </u>			
38	103	104	159	232	236	245	248	252	 	 	 	
38	76	103	104	159	213	232	236	245	260		 	
68	76	103	104	159	213	232	236	245	260	271	-	
68	76	103	104	159	209	213	232	236	245	260	ļ	
68	76	103	104	159	210	213	232	236	245	260	ļ	
68	76	103	104	159	205	213	232	236	245	260	 	+
68	76	103	104	159	210	232	236	245	260		 	-
68	103	104	159	213	232	236	245	260	 	 		
76	103	104	159	213	232	236	245	260				
68	103	104	159	209	232	236	245	 	-		-	_
68	103	3 104	159	210	232	236	245	+	-		+	
68	10:	3 104	159	230	232	236	245	-			 	
68	10	3 104	159	126	232	236	245			-		
68	3 10	3 104	1 159	205	232	236	245				-	
6	3 10	3 10-	4 159	210	232	236	245	-	-		-	
10	3 10	4 15	9 230	236	245	 		_			+-	
6	В 10	3 10	4 159	232	236	245	260		_			 -
10	3 10	4 15	9 232	2 236	245							
6	8 10	3 10	4 159	174	232	236	245	25	7			
6	8 10	3 10	4 159	9 194	232	236	245	25	7			
6	8 10	3 10	4 159	9 209	232	236	245	25	7			

103	104	159	232	236	245	257						
		103		159	213		226	245	260	261		
68	76		104			232	236	245	260	261		
68	103	104	159	232	236	245	257	261				
103	104	159	213	232	236	245	260					
103	104	159	210	232	236	245	248	252				
103	104	159	209	232	236	245	257					
68	76	103	104	159	210	213	232	236	245	260		
12	103	104	159	209	213	232	236	245	260			
103	104	209	232	236	245	257						
103	104	159	205	210	213	232	236	245	260			
103	104	159	205	209	232	236	245	260				
68	103	104	159	205	209	210	232	236	245			
103	104	159	205	209	210	232	236	245	257			
103	104	159	205	209	232	236	245	257				
68	103	104	159	205	209	210	232	236	245	260		
103	104	159	205	209	210	232	236	245				
103	104	159	209	210	232	236	245					
103	104	159	205	210	232	236	245					
68	103	104	128	159	232	236	245					
48	103	104	159	230	236	245						
48	68	103	104	159	209	232	236	245				
48	68	103	104	159	232	236	245	248	252			
48	68	103	104	159	232	236	245	257	261			
102	103	104	159	212	232	236	245	248	252			
12	102	103	104	159	212	232	236	245	248	252		
101	102	103	104	159	212	232	236	245	248	252		
98	102	103	104	159	212	232	236	245	248	252		
102	103	104	159	213	232	236	245	248	252			
103	104	131	159	232	236	245	248	252				
103	104	159	184	232	236	245	248	252				
103	104	159	232	236	244	245	248	252				
62	103	+	159	213	232	236	245	248	252	256		
12	62	103	104	159	213	232	236	245	248	252		
101	103	104	159	185	232	236	245	248	252	T		
101	103	104	159	206	232	236	245	248	252	<u> </u>		1
101	103	—	159	213	232	236	245	248	252		 	

98	102	103	104	159	232	236	245	248	252			
101	102	103	104	159	232	236	245	248	252			
98	102	103	104	159	212	232	236	245	248	252		
98	102	103	104	159	212	232	236	248	252			
62	103	104	109	159	213	232	236	245	248	252		
62	103	104	159	212	213	232	236	245	248	252		
62	101	103	104	159	212	213	232	236	245	248	252	
103	104	159	232	245	248	252						
103	104	159	230	245								
62	103	104	130	159	213	232	236	245	248	252		
101	103	104	130	159	232	236	245	248	252			
101	103	104	128	159	232	236	245	248	252			
62	101	103	104	159	213	232	236	245	248	252		
62	103	104	128	159	213	232	236	245	248	252		
62	103	104	128	159	213	232	236	245	248	252		
101	103	104	159	232	236	245	248	252	260			
101	103	104	131	159	232	236	245	248	252			,
98	101	103	104	159	232	236	245	248	252			
99	101	103	104	159	232	236	245	248	252			:
101	103	104	159	212	232	236	245	248	252			
101	103	104	159	209	232	236	245	248	252			
101	103	104	159	210	232	236	245	248	252			
101	103	104	159	205	232	236	245	248	252			
101	103	104	159	230	236	245						
101	103	104	159	194	232	236	245	248	252			
76	101	103	104	159	194	232	236	245	248	252		
101	103	104	159	230	232	236	245	248	252			
62	103	104	159	185	206	213	232	236	245	248	252	271

An even more preferred protease variant useful in the cleaning compositions of the present invention include a substitution set (one substitution set per row in the following Table VII) selected from the group consisting of:

		_			Ta	ble VII	·	 	
N76D	S103A	V104I	S212P	E271V					

N76D	S103A	V1041	N252K	N261Y								
N76D_	S103A	V1041	S212P	G258R								
V4E	N76D	S103A	V104I	G159D	L217E	N252D					ļ	
Q12H	N62H	N76D	S103A	V1041	G159D							
N76D	S103A	V1041	S212P	V268F	E271V						<u> </u>	
N76D	S87R	S103A	V1041	S212P	E271V						 	
N76D	S103A	V1041	S212P	Q245L	E271V	<u> </u>					-	
N76D	S103A	V1041	T134S	S141N	S212P	E271V					 	
N76D	S103A	V104I	S212P	Q236L	N243S	E271V					 	
G20V	N62S	N76D	S103A	V1041							ļ	
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R				<u> </u>	
N76D	S103A	V104I	A232V	Q245R							<u> </u>	-
S24T	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R			 	
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N252K				 	
V68A	N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			<u> </u>	
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R					 	
V68A	S103A	V104I	N140D	G159D	A232V	Q236H	Q245R	N252K				
N43S	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N252K				
N43K	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	<u> </u>				
N43D	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N252K	<u> </u>	ļ	_	
V68A	S87G	S103A	V104I	G159D	A232V	Q236H	Q245R	N252K	R275S	 		
V68A	\$103	4 V104I	G159D	A232V	Q236H	Q245R	L257V	ļ		<u> </u>		
V68A	\$103	4 V104I	N116D	G159D	A232V	Q236H	Q245R		ļ	ļ		\dashv
V68A	S103	A V104I	G159D	A232V	Q236H	Q245R	N248D			<u> </u>		
R10C	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R		ļ	<u> </u>		
V68A	"	A V104I	G159D	V203E	A232V	Q236H	Q245R	<u> </u>			+	
V68A		A V1041		A232V	Q236H	K237E	Q245R	 		 		
V68A			S103A	V104I	G159D	A232V	Q236H	Q245R		_		
V68A	\$103	A V104	G159I	N183E	A232V	Q236H	Q245R					_
V68A		A V104		A174\	Q206L	. A232V	Q236H	Q245R				
V68/		A V104		S188C	A232V	Q236H	Q245R			-		-
V68/		A V104	G1591	A2301	A232\	/ Q236H	Q245R					
V68/				G1591) A232\	/ Q236H	Q245R					
V68		3A V104		D A215	F A232	/ Q236H	Q245R					
V68.		3A V104					N248S					
بوون												

V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R					
V68A	N76D	S103A	V104I	G159D	P210R	A232V	Q236H	Q245R				
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V				
N76D	S103A	V104I	A232V	Q236H	Q245R	L257V						
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V	R275H				
N76D	S103A	V1041	L257V	R275H								
V68A	S103A	V104I	G159D	T224A	A232V	Q236H	Q245R	L257V				
N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	1.257V					
V68A_	N76D	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R				
V68A	N76D	S103A_	V104I	G159D	G211R	A232V	Q236H	Q245R				
V68A	N76D	S103A	V104I	G159D	G211V	A232V	Q236H	Q245R				
Q12R_	V68A	N76D	S103A	V104I	G159D	Y214L	A232V	Q236H	Q245R			
V68A	N76D	S103A	V104I	G159D	A215R	A232V	Q236H	Q245R				
Q12R	V68A	N76D	S103A	V1041	G159D	A232V	Q236H	Q245R				
G20R	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	S259G			
V68A	S87R	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	T260V			
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N261G				
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N261W				L
N76D	S103A	V104I	A232V	Q236H	S242P	Q245R						
V68A	N76D	S103A	V104I	G159D	P210L	A232V	Q236H	Q245R				ļ
Q12R	A48V	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R			
N76D	S103A	V104I	A232V	Q236H	Q245R							
N76D_	S103A	V104I	G159D	Y192F	A232V	Q236H	Q245R					
N76D	S103A	V104I	V1471	G159D	A232V	Q236H	Q245R	N248S	K251R			
Q12R	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	A272S			<u> </u>
V68A	N76D	S103A	V104I	G159D	N183K	Q206L	A232V	Q236H	Q245R			<u> </u>
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	S256R				
V68A	N76D	S103A	V1041	G159D	Q206R	A232V	Q236H	Q245R			ļ	<u> </u>
K27R	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R				<u> </u>
V68A	N76D	S103A	V104I	N116T	G159D	R170S	N185S	A232V	Q236H	Q245R		<u> </u>
G61E	V68A	\$103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	<u></u>		
N43D	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212P	A232V	Q236H	Q245R	N248D	N252K			
V68A		V104I	S99N	G159D	N184D	A232V	Q236H	Q245R	N248D	N252K		
S103A		G159D	A232V	Q236H	Q245R							
V68A		V104I	G159D	Y209W		Q236H		T	N252K			
1.55.			,	<u> </u>								

V68A	S103A	V104I	Q109R	G159D	A232V	Q236H	Q245R	N248D	N252K			
G20R	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	Y209F	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	N261D_			
V68A	S103A	V104I	G159D	N185D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	P210R	A232V	Q236H_	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	P210T	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	P210S	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	N185D	P210L	A232V	Q236H	Q245R	N248D	N252K		
V68A	S103A	V104I	G159D	P210L	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212A	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S212E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	T213E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	T213S	A232V	Q236H	Q245R	N248D	N252K				
V68A	A103V	V104I	G159D	T213E	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	T213G	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A215V	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A215R	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S216T	A232V	Q236H	Q245R	N248D	N252K			
V68A_	S103A	V104I	G159D	S216V	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	S216C	A232V	Q236H	Q245R	N248D	N252K	<u> </u>	ļ	
G20A	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V1041	G159D	N173D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	K251V	N252K			
V68A	S103A	V104I	G159D	Q206R	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252F	ļ			ļ
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252L		-	ļ	ļ
P55S	V68A	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252F	ļ		
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	T255V			ļ
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	S256N	<u> </u>		
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	S256E	ļ		 -
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	S256R		<u> </u>	
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	T260R	ļ	<u> </u>	
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	L257R	<u> </u>	<u> </u>	<u> </u>

V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	G258D			
18V	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	N269D		
V68A	S103A	V104l	N116S	G159D	A232V	Q236H	Q245R	N248D	N252K	T260E		
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R_	N248D	N252K	N261R			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	N261D			
V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	A232V	Q236H	Q245R	N248D	N252K					
S103A	V104I	G159D	A232S	Q236H	Q245R	N248D	N252K					
V68A	S103A	V104I	G159D	A232V	Q236R	Q245R	N248D	N252K				
N18S	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245V	N248D	N252K				
V68A	N76D	S101T	S103A	V104I	G159D	T213R	N218S	A232V	Q236H	Q245R	T260A	
V68A	S103A	V104I	G159D	A228V	A232V	Q236H	Q245R	N248D	N252K			
T33S	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K		
V68A	N76D	E89D	S103A	V104I	G159D	P210L	T213R	A232V	Q236H	Q245R	T260A	
G61E	V68A	N76D	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K		
S103A	V104I	G159D	V2051	P210I	A232V	Q236H	Q245R					
G61E	V68A	S103A	V1041	S130A	G159D	A232V	Q236H	Q245R	N248D	N252K		
G61E	V68A	S103A	V104I	A133S	Q137R	G159D	A232V	Q236H	Q245R	N248D	N252K	
G61E	S103A	V104I	A133V	G159D	A232V	Q236H	Q245R	N248D	N252K			
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248G	N252K				
V68A	S103A	V104i	G159D	N218S	A232V	Q236H	Q245R	N248D	N252K			
G61E	V68A	S103A	V104I	G159D	S160V	A232V	Q236H	Q245R	N248D	N252K		
S3L	G61E	V68A	N76D	S103A	V104I	A232V	Q236H	Q245R	N248D	N252K		
G61E_	V68A	S103A	V104I	G159D	S167F	A232V	Q236H	Q245R	N248D	N252K		
G97E	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	<u> </u>	ļ <u>.</u>		
A98D	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K				
S99E	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	<u> </u>	<u> </u>	<u> </u>	
S101E	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				ļ
S101G	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K				
G102A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				<u> </u>
S103A	V104I	S106E	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V104I	Q109E	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K	N261R				
S103A	V104I	Q109R	G159D	A232V	Q236H	Q245R	N248D	N252K				
N62D		V104I	G159D	A232V	Q236H	Q245R	N248D	N252K				

S103A	V104I	G159D	N184D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	G159D	S166D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	G159D	L217E	A232V	Q236H	Q245R	N248D	N252K			
G20R	N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K	
N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S103A	V104I	G159D	Q206R	L217E	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V1041	G159D	Q206R	A232V	Q236H	Q245R	N248D	N252K		
S103A	V104I	\$130G	G159D	A232V	Q236H	Q245R	N248D	N252K			
S103A	V1041	P131V	G159D	A232V	Q236H	Q245R	N248D	N252K			
K27N	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
T38G	S103A	V1041	G159D	A232V	Q236H	Q245R	N248D	N252K			
T38A	N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A		
V68A	N76D	\$103A	V1041	G159D	T213R	A232V	Q236H	Q245R	T260A	E271G	
V68A	N76D	S103A	V104I	G159D	Y209W	T213R	A232V	Q236H	Q245R	T260A	
V68A	N76D	S103A	V104I	G159D	P210I	T213R	A232V	Q236H	Q245R	T260A	
V68A	N76D	\$103A	V1041	G159D	V2051	T213R	A232V	Q236H	Q245R	T260A	
V68A	N76D	S103A	V104I	G159D	P210I	A232V	Q236H	Q245R	T260A		
V68A	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A			
N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A			
V68A	S103A	V1041	G159D	Y209W	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	P210I	A232V	Q236H	Q245R				
V68A	S103A	V1041	G159D	A230V	A232V	Q236H	Q245R				
V68A	S103A	V1041	G159D	L126F	A232V	Q236H	Q245R				
V68A	S103A	V104I	G159D	V2051	A232V	Q236H	Q245R				
V68A	S103A	V1041	G159D	P210L	A232V	Q236H	Q245R				
S103A	V1041	G159D	A230V	Q236H	Q245R	L					
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	T260A				
S103A	V1041	G159D	A232V	Q236H	Q245R						
V68A	S103A	V104I	G159D	A174V	A232V	Q236H	Q245R	L257V			
V68A	S103A	V104I	G159D	A194S	A232V	Q236H	Q245R	L257V			
V68A	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	L257V			
S103A	V104I	G159D	A232V	Q236H	Q245R	L257V					
V68A	N76D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A	N261W	
V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V	N261W			
S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	T260A	<u> </u>			
S103A	V104I	G159D	P210I	A232V	Q236H	Q245R	N248D	N252K			

		Ţ		<u> </u>	Γ	T	<u> </u>			Τ	Γ	
S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	L257V	 		ļ <u> </u>		
V68A	N76D	S103A	V1041	G159D	P210L	T213R	A232V	Q236H	Q245R	T260A		
Q12R	S103A	V104I	G159D	Y209W	T213R	A232V	Q236H	Q245R	T260A			
S103A	V104I	Y209W	A232V	Q236H	Q245R	L257V						
S103A	V104I	G159D	V2051	P210I	T213R	A232V	Q236H	Q245R	T260A			
S103A	V1041	G159D	V2051	Y209W	A232V	Q236H	Q245R	T260A				
V68A	S103A	V104I	G159D	V2051	Y209W	P2101	A232V	Q236H	Q245R			
S103A	V104I	G159D	V2051	Y209W	P210I	A232V	Q236H	Q245R	L257V			
S103A	V104I	G159D	V2051	Y209W	A232V	Q236H	Q245R	L257V				
V68A	S103A	V104I	G159D	V2051	Y209W	P210I	A232V	Q236H	Q245R	T260A		
S103A	V104I	G159D	V205I	Y209W	P210I	A232V	Q236H	Q245R				
S103A	V104I	G159D	Y209W	P210I	A232V	Q236H	Q245R					
S103A	V104I	G159D	V2051	P2101	A232V	Q236H	Q245R					
V68A	S103A	V104I	S128L	G159D	A232V	Q236H	Q245R					
A48V	S103A	V104I	G159D	A230V	Q236H	Q245R						
A48V	V68A	S103A	V1041	G159D	Y209W	A232V	Q236H	Q245R				
A48V	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
A48V	V68A	S103A	V104I	G159D	A232V	Q236H	Q245R	L257V	N261W			
G102A	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K			
Q12R	G102A	S103A	V1041	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K		
S101G	G102A	S103A	V1041	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K		
A98L	G102A	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K		
G102A	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K			
S103A	V104I	P131V	G159D	A232V	Q236H	Q245R	N248D	N252K				
S103A	V1041	G159D	N184S	A232V	Q236H	Q245R	N248D	N252K				
S103A	V104I	G159D	N184G	A232V	Q236H	Q245R	N248D	N252K				
S103A	V104I	G159D	A232V	Q236H	V244T	Q245R	N248D	N252K				
S103A	V104I	G159D	A232V	Q236H	V244A	Q245R	N248D	N252K				
N62D	S103A	V104I	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K	S256R		
Q12R	N62D	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S101G	S103A	V104I	G159D	N185D	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	G159D	Q206E		Q236H	Q245R	N248D	N252K			
S101G	S103A	V1041	G159D	T213Q		Q236H	Q245R	N248D	N252K			
A98L	G102A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G	G102A	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
A98L	G102A		V1041	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K		
						· ·	4-2011	· · ·				

A98L	G102A	\$103A	V104I	G159D	S212G	A232V	Q236H	N248D	N252K			
N62D	S103A	V104I	Q109R	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V104I	G159D	S212G	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S101G	S103A	V1041	G159D	S212G	T213R	A232V	Q236H	Q245R	N248D	N252K	
S103A	V1041	G159D	A232V	Q245R	N248D	N252K						
S103A	V104I	G159D	A230V	Q245R								
N62D	S103A	V1041	S130G	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S101G	S103A	V1041	S130G	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	S128G	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	S128L	G159D	A232V	Q236H	Q245R	N248D	N252K			
N62D	S101G	S103A	V1041	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V104I	S128G	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
N62D	S103A	V1041	S128L	G159D	T213R	A232V	Q236H	Q245R	N248D	N252K		
S101G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K	T260A			
S101G	S103A	V104I	P131V	G159D	A232V	Q236H	Q245R	N248D	N252K			
A98V	S101G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S99G	S101G	S103A	V104I	G159D	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	G159D	S212G	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V104I	G159D	Y209W	A232V	Q236H	Q245R	N248D	N252K			
S101G	S103A	V1041	G159D	P210I	A232V	Q236H	Q245R	N248D	N252K			ļ
S101G	S103A	V104I	G159D	V2051	A232V	Q236H	Q245R	N248D	N252K			<u> </u>
S101G	S103A	V104I	G159D	A230V	Q236H	Q245R						
S101G	S103A	V104I	G159D	A194P	A232V	Q236H	Q245R	N248D	N252K			
N76D	S101G	\$103A	V104I	G159D	A194P	A232V	Q236H	Q245R	N248D	N252K		
S101G	S103A	V1041	G159D	A230V	A232V	Q236H	Q245R	N248D	N252K			
N62D	S103A	V1041	G159D	N185D	Q206E	T213R	A232V	Q236H	Q245R	N248D	N252K	E271Q

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table VI except for the following substitution set of Table VIII:

					T	able VII	l ·			
68	76	103	104	116	159	170	185	232	236	245

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table IX:

~	1 1	**7
l a	ble	: I X

							Table l	\mathbf{X}			
68	103	104	159	232	236	245	252			T	
68	76	103	104	159	213	232	236	245	260	1	
68	103	104	159	232	236	245	248	252	 		
68	103	104	159	232	236	245		1		+	
68	103	104	140	159	232	236	245	252	 	1	
43	68	103	104	159	232	236	245	252			
43	68	103	104	159	232	236	245				
68	103	104	159	232	236	245	257		 		
68	76	103	104	159	210	232	236	245		 	
68	103	104	159	224	232	236	245	257		 	
76	103	104	159	232	236	245	257			1	
68	76	103	104	159	211	232	236	245			
12	68	76	103	104	159	214	232	236	245	†	
68	76	103	104	159	215	232	236	245			
12	68	76	103	104	159	232	236	245			
20	68	76	103	104	159	232	236	245	259		
68	76	87	103	104	159	232	236	245	260		
68	76	103	104	159	232	236	245	261			
12	48	68	76	103	104	159	232	236	245		
76	103	104	159	192	232	236	245				†
76	103	104	147	159	232	236	245	248	251		
12	68	76	103	104	159	232	236	245	272		
68	76	103	104	159	183	206	232	236	245		
68	76	103	104	159	232	236	245	256			
68	76	103	104	159	206	232	236	245			
27	68	76	103	104	159	232	236	245			1
68	103	104	159	212	232	236	245	248	252		†
103	104	159	232	236	245	248	252				

									(61									\neg
						200	π.	232	230	6 1 3	245		248	7 2	252				
8	103	3	104	1:	59	209				_	245		248	+	252				1
58	10:	<u>3</u> †	104	1	09	159	9	232	23			4					一十		ヿ
	<u> </u>	_	103	+-	04	15	9	232	23	6	24:	5	248		252		}		ᅱ
20	68	_		4	159	20	19	232	23	36	24	5	24	B	252				_
68	10	3	104						12	36	24	5	24	8	252		1		
68	10)3	104	1	159	2.	10	232					24	•	252	1			
68	+-	03	10	4	159	2	12	232	2	36	24	• >				┼			
	4-		10		159	2	13	232	2	36	2	45	24	18	252	1-		├	
68		03		_		+-	232	236	: 1:	245	2	48	2	52				<u> </u>	
68] 1	03	10	14	213	1_		232	-	236	1-2	45	1 2	48	252				
68	3	103	10	04	159		215				1	245	+	48	252	十		T	
6	2	103	1	04	159		216	23	2	236	1_		1		252	}-		+	
L	_	68		03	104	+	159	23	2	236		245		248	L			╫	
2	0				159	\dashv	232	1 23	6	245	1	248		252	255			\bot	
1	8	10	3]	104		_		4_	36	245	+	248	1	252	256	5			
1	68	10	13	104	15	9	232				_	241	-	252	260	5		\top	
+	68	10	13	104	15	9	232	2 2	36	24:	_		_		25			+	
-			03	104	15	9	22	8 2	32	23	6	24	5	248	1		24:	+	26
L	68				4	03	10	4 1	59	21	0	21	3	232	23	0			
1	68		16	89					232	123	36	24	15	248	25	52			
t	68	1	03	104	4 1	59 		10											

Still yet an even more preferred protease variant useful in the cleaning composition of the present invention include a substitution set selected from the group consisting of the substitution sets in Table X:

bstituti	on sets	s in	Iadio	. A.		7	Table X				NY252K		
		3.73	OAT TO	3159D	A228V	A232V	O236H	Q245F	N	248D	NZSZK		
V68A S							Q236H	Q2451	2 1	1248D			
V68A S	3103A			لسنا			Q236H		_	V248D	N252K		77004
G20R	V68A	SI	03A	V104I		<u> </u>	4			A232V	Q236H	Q245R	T260A
V68A	N76D	E	89D	S103A	·	1	Q245I	1 .		N252K	S256R		
V68A	S103A	V	104I	G159D	1					N252K	1		
V68A		_	1041	G159D	A232\		Q245					+	
V68A	L	1_	/104I	G159I	A232	V Q236I	H Q245	R N24			1		+
1	·	1	/104I		A232	V Q236	H Q245	R N24	8D	N2521		`	
V68A	1				D A232		H Q24	R N2	8D	N252	<u> </u>		
V68A	S103		V1041					6H Q2	45R	N248	D N252	K	
V68A	S103	A	V104					6H 02	45P	N248	D N252	K	
V68A	S103	3A	V104	I G159	D A21	N A23.	2 1 023	6H 02	451	N24	N252	K	
1	A S10	_	V104	I G159	D A21	5R A23	2V Q23	ori Q					

т			16:	EOD I	50101	A232V	1022	<u> </u>	02451	2 N	24801	N252k	. .			\neg
V68A					P210L		1			I	1	N2521				
V68A	S103A	V10			S212C	A232V	1	1		- 1						\dashv
V68A	S103A	1	1 _		S212G	A232V	1	- 1		L_	2480	N2521	_			\dashv
S103A	V104	G15	9D A	1	Q236H		1	1			2010	212521				
V68A	S103/	V10	- 1		Y209W		1			•					ļ	\dashv
V68A	S103	V10	041 Q	109R	G159D		1	·		- 1		ŀ			ļ	
G20R	V68/	S10	3A \	/1041	G159D	1		1		- 1						
V68A	S103.	A VI	041 G	159D	Y209F	A232V					1248D	N252	K		<u> </u>	
N76D	S103	A VI	041 C	159D	Y192F	1	ľ		1	- 1					1_	
N76D	S103	A VI	041	V1471	G159D	1	- 1		1	- 1		1			_	
Q12R	V68.	A N7	6D S	3103A	V1041	G159I) A2	32V	Q236	1	_	A272				
V68A	N76	D S10	03A \	V1041	G159D	N1831	K Q2	206L	A232	2V (Q236H	Q24:	5R			
V68A	N76	D SI	03A	V104I	G159D	A232	V Q2	236H	Q24	1	S256R					
V68A	N76	D SI	03A	V1041	G159E	Q206	R A2	232V	Q23	6H	Q245R				1_	
K27R	V68	AN	76D	S103A	V1041	G159	D A	232V	Q23	- 1	Q245F					
Q12F	A48	V V	68A	N76D	S103A	V104		159D	1	Į.		I Q24	5R		\perp	
V68/	N76	D SI	03A	V1041	G159I	A232	V Q	236F	1 .		N261V				\bot	
V68/	N70	D SI	03A	V104	G1591	D G211	R A	232\	/ Q23	6H	Q2451	2			\perp	
V68/	A N7	5D S1	03A	V104	G1591	D G211	VA	232\	/ Q23	36H	Q245	_1				
Q12	R V6	8A N	76D	S103/	V104	I G159	DY	2141	L A2:	32V	Q236	H Q24	15R		\perp	
V68.			103A	V104	I G159	D A21:	5R A	232	V Q2:	36H	Q245	R				
Q12	R V6	8A N	176D	S103	A V104	I G15	9D A	232	V Q2	36H	Q245	R			\perp	
G20		8A N	176D	S103	A V104	11 G15	9D A	232	V Q2	36H	Q245	R S2:	59G			
V68		6D S	587R	S103.	A V104	41 G15	9D A	232	V Q2	36H	Q245	R T2	60V		\bot	
N76	D SI	03A \	/104I		D A232				R L2							
V68	A N	76D S	103A		I G159								60A			
V68	AN	76D S	5103A	V10	41 G159	9D P21	OR A	A232	V Q2	236H	Q24:	5R				
V61	A SI	03A	V104I	G159	D S21	2P A23	32V	Q236	H Q	245R	N24	BD N2	252K			
	- 1	03A			D T22	4A A2	32V	Q236	SH Q	245F	L25	7V				
	- 1				D A23	2V Q2	36H	Q24:	SR N	2529	3					
	1	103A			9D A23		36H	Q24	5R N	252l						
	- 1	103A		1	9D A23	1	36H	Q24	5R N	2481	N25	2K				
		103A		1	9D A23		36H	Q24	5R		1	_		\top		
l l	1	103A		1	0D G1:					245	R N25	2K		1		
1	i_		S103/			59D A2		•								
		768A				59D A2		1						1		
LIN2	131	, von		11.				<u> </u>								

							002(11	O245D	N252K			
г	N/42D	V684	S103A	V1041	G159D	A232V	Q236H	Q243K	1423214	\		l
- 1	19430	V 00/1	0.00.1				0245P	1 2577			ļ !	١
H	1/68 A	S103A	V104I	G159D	A232V	Q236H	QZ43K	12271	1	_	 	j
- 1	V 007	510511		l		<u> </u>	1	·				
1												

A highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12/102/103/104/159/212/232/236/245/248/252; 61/68/103/104/159/232/236/245/248/252; 62/103/104/130/159/213/232/236/245/248/252; 62/103/104/159/213/232/236/245/248/252; 62/103/104/109/159/213/232/236/245/248/252; 62/103/104/159/232/236/245/248/252; 62/101/103/104/159/212/213/232/236/245/248/252; 68/103/104/159/232/236/245/248/252/270; 68/103/104/159/210/232/236/245/248/252; 68/103/104/159/185/232/236/245/248/252; 68/103/104/159/185/210/232/236/245/248/252; 68/103/104/159/213/232/236/245/248/252; 68/76/103/104/159/209/232/236/245; 68/103/104/159/230/232/236/245; 68/103/104/213/232/236/245/248/252; 68/103/104/232/236/245/248/257/275; 68/103/104/159/209/232/236/245; 68/103/104/159/232/236/245/248/252; 68/103/104/159/232/236/245/252; 68/76/103/104/159/232/236/245; 68/103/104/159/232/236/245/257; 68/103/104/159/232/236/245; 68/76/103/104/159/215/232/236/245; 68/76/103/104/159/211/232/236/245; 68/103/104/159/213/232/236/245/260; 68/103/104/159/210/232/236/245; 68/76/103/104/159/210/232/236/245/260; 68/76/103/104/159/213/232/236/245/260; 68/103/104/232/236/245/257/275; 68/103/104/159/183/232/236/245/248/252; 76/103/104/159/232/236/245; 68/103/104/159/213/232/236/245; 76/103/104/131/159/232/236/245/248/252; 76/103/104/159/213/232/236/245/260; 98/103/104/159/232/236/245/248/252; 97/103/104/159/232/236/245/248/252; 98/102/103/104/159/212/232/236/245/248/252; 101/103/104/159/232/236/245/248/252; 103/104/159/232/236/245; 102/103/104/159/232/236/245/248/252; 103/104/159/232/236/245/248/252; 103/104/159/248/252/270; 103/104/159/232/245/248/252; 103/104/159/205/209/232/236/245/257 103/104/159/213/232/236/245/248/252; 103/104/159/205/209/210/232/236/245/257; 103/104/130/159/232/236/245/248/252; 103/104/159/217/232/236/245/248/252; 103/104/159/205/209/232/236/245; and 103/104/131/159/232/236/245/248/252; 103/104/159/232/236/245/257.

A more highly preferred protease variant useful in the cleaning compositions of the present invention include a substitution set selected from the group consisting of:

12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 61E/68A/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K; 62D/103A/104I/159D/213R/232V/236H/245R/248D/252K; 62D/103A/104I/159D/232V/236H/245R/248D/252K; 62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K; 62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K; 68A/76D/103A/1041/159D/213R/232V/236H/245R/260A; 68A/76D/103A/104I/159D/210I/232V/236H/245R/260A; 68A/103A/104I/159D/183D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/209W/232V/236H/245R; 68A/76D/103A/104I/159D/211R/232V/236H/245R; 68A/76D/103A/104I/159D/215R/232V/236H/245R; 68A/103A/104I/159D/213R/232V/236H/245R/260A; 68A/76D/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/252K; 68A/103A/104I/159D/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/257V; 68A/103A/104I/159D/185D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K; 68A/103A/104I/159D/213E/232V/236H/245R/248D/252K; 68A/103A/1041/159D/230V/232V/236H/245R; 68A/76D/103A/104I/159D/209W/232V/236H/245R; 68A/103A/104I/232V/236H/245R/248D/257V/275H; 68A/103A/1041/232V/236H/245R/257V/275H; 68A/103A/104I/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210I/232V/236H/245R; 68A/103A/104I/159D/210L/232V/236H/245R; 68A/103A/104I/159D/213G/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/248D/252K/270A; 76D/103A/104I/159D/232V/236H/245R; 76D/103A/104I/131V/159D/232V/236H/245R/248D/252K; 76D/103A/104I/159D/213R/232V/236H/245R/260A; 97E/103A/104I/159D/232V/236H/245R/248D/252K; 98L/103A/104I/159D/232V/236H/245R/248D/252K;

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98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;
101G/103A/104I/159D/232V/236H/245R/248D/252K;
102A/103A/104I/159D/232V/236H/245R/248D/252K;
103A/104I/159D/213R/232V/236H/245R/248D/252K;
103A/104I/159D/213R/232V/236H/245R/248D/252K;
103A/104I/130G/159D/232V/236H/245R/248D/252K;
103A/104I/159D/217E/232V/236H/245R/248D/252K;
103A/104I/159D/217E/232V/236H/245R/248D/252K;
103A/104I/159D/232V/236H/245R;
103A/104I/159D/205I/209W/232V/236H/245R;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/232V/236H/245R/257V;
103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and

Recombinant Proteases/Recombinant Subtilisins - A "recombinant protease" or "recombinant subtilisin" refers to a protease or subtilisin in which the DNA sequence encoding the naturally-occurring protease or subtilisin, respectively, is modified to produce a mutant DNA sequence which encodes the substitution, insertion or deletion of one or more amino acids in the protease or subtilisin amino acid sequence. Suitable modification methods are disclosed herein, and in U.S. Patent Nos. RE 34,606, 5,204,015 and 5,185,258.

Non-Human Proteases/Non-Human Subtilisins - "Non-human proteases" or "non-human subtilisins" and the DNA encoding them may be obtained from many procaryotic and eucaryotic organisms. Suitable examples of procaryotic organisms include gram negative organisms such as *E. coli* or *Pseudomonas* and gram positive bacteria such as *Micrococcus* or *Bacillus*. Examples of eucaryotic organisms from which carbonyl hydrolase and their genes may be obtained include yeast such as *Saccharomyces cerevisiae*, fungi such as *Aspergillus* sp. and non-human mammalian sources such as, for example, fungi such as *Aspergillus* sp. and non-human mammalian sources such as, for example, bovine sp. from which the gene encoding the protease chymosin or subtilisin chymosin can be obtained. A series of proteases and/or subtilisins can be obtained from various related species which have amino acid sequences which are not entirely homologous between the members of that series but which nevertheless exhibit the same or similar type of biological activity. Thus, non-human protease or non-human subtilisin as used herein have a functional definition which refers to proteases or subtilisins, respectively, which are associated, directly or indirectly, with procaryotic and eucaryotic sources.

Variant DNA Sequences - Variant DNA sequences encoding such protease or subtilisin variants are derived from a precursor DNA sequence which encodes a naturallyoccurring or recombinant precursor enzyme. The variant DNA sequences are derived by modifying the precursor DNA sequence to encode the substitution of one or more specific amino acid residues encoded by the precursor DNA sequence corresponding to positions 103 in combination with one or more of the following positions 1, 3, 4, 8, 10, 12, 13, 15, 16, 17, 18, 20, 21, 22, 24, 25, 27, 33, 37, 38, 42, 43, 48, 55, 57, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 115, 116, 117, 119, 121, 123, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 161, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin. Although the amino acid residues identified for modification herein are identified according to the numbering applicable to B. amyloliquefaciens (which has become the conventional method for identifying residue positions in all subtilisins), the preferred precursor DNA sequence useful for the present invention is the DNA sequence of Bacillus lentus as shown in Fig. 3.

In a preferred embodiment, these variant DNA sequences encode the substitution, insertion or deletion of the amino acid residue corresponding to position 103 of *Bacillus amyloliquefaciens* subtilisin in combination with one or more additional amino acid residues corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions

corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of *Bacillus amyloliquefaciens* subtilisin. More preferably, these variant DNA sequences encode the protease variants described herein.

In another preferred embodiment, these variant DNA sequences encode the substitution, insertion or deletion of one or more of the amino acid residues corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin. More preferably, these variant DNA sequences encode the protease variants described herein.

Although the amino acid residues identified for modification herein are identified according to the numbering applicable to *B. amyloliquefaciens* (which has become the conventional method for identifying residue positions in all subtilisins), the preferred precursor DNA sequences useful for the present invention is the DNA sequence of *Bacillus lentus* as shown in Fig. 3.

These recombinant DNA sequences encode protease variants having a novel amino acid sequence and, in general, at least one property which is substantially different from the same property of the enzyme encoded by the precursor protease DNA sequence. Such properties include proteolytic activity, substrate specificity, stability, altered pH profile and/or enhanced performance characteristics.

Specific substitutions corresponding to positions 103 in combination with one or more of the following positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a subtitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 wherein the numbered positions correspond to the naturallyoccurring subtilisin from Bacillus amyloliquefaciens or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins (such as Bacillus lentus subtilisin) are described herein. Further, specific substitutions corresponding to one or more of the following positions 62, 212, 230, 232, 252 and 257 wherein the numbered positions correspond to the naturally-occurring subtilisin from Bacillus amyloliquefaciens or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins (such as Bacillus lentus subtilisin) are

described herein. These amino acid position numbers refer to those assigned to the mature Bacillus amyloliquefaciens subtilisin sequence presented in Fig. 1. The present invention, however, is not limited to the use of mutation of this particular subtilisin but extends to precursor proteases containing amino acid residues at positions which are "equivalent" to the particular identified residues in Bacillus amyloliquefaciens subtilisin. In a preferred embodiment of the present invention, the precursor protease is Bacillus lentus subtilisin and the substitutions, deletions or insertions are made at the equivalent amino acid residue in B. lentus corresponding to those listed above.

A residue (amino acid) of a precursor protease is equivalent to a residue of *Bacillus amyloliquefaciens* subtilisin if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific residue or portion of that residue in *Bacillus amyloliquefaciens* subtilisin (i.e., having the same or similar functional capacity to combine, react or interact chemically).

In order to establish homology to primary structure, the amino acid sequence of a precursor protease is directly compared to the *Bacillus amyloliquefaciens* subtilisin primary sequence and particularly to a set of residues known to be invariant in subtilisins for which sequence is known. For example, Fig. 2 herein shows the conserved residues as between *B. amyloliquefaciens* subtilisin and *B. lentus* subtilisin. After aligning the conserved residues, allowing for necessary insertions and deletions in order to maintain alignment (i.e., avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of *Bacillus amyloliquefaciens* subtilisin are defined. Alignment of conserved residues preferably should conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues. Conservation of the catalytic triad, Asp32/His64/Ser221 should be maintained.

For example, in Fig. 3 the amino acid sequence of subtilisin from Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis (carlsbergensis) and Bacillus lentus are aligned to provide the maximum amount of homology between amino acid sequences. A comparison of these sequences shows that there are a number of conserved residues contained in each sequence. These conserved residues (as between BPN' and B. lentus) are identified in Fig. 2.

These conserved residues, thus, may be used to define the corresponding equivalent amino acid residues of *Bacillus lentus* (PCT Publication No. WO89/06279 published July 13, 1989), the preferred protease precursor enzyme herein, or the subtilisin referred to as PB92 (EP 0 328 299), which is highly homologous to the preferred *Bacillus lentus* subtilisin. The amino acid sequences of certain of these subtilisins are aligned in Figs. 3A and 3B with the sequence of *Bacillus amyloliquefaciens* subtilisin to produce the maximum

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homology of conserved residues. As can be seen, there are a number of deletion in the sequence of Bacillus lentus as compared to Bacillus amyloliquefaciens subtilisin. Thus, for example, the equivalent amino acid for Val165 in Bacillus amyloliquefaciens subtilisin in Thus, for example, the other subtilisins is isoleucine for B. lentus and B. licheniformis. the amino acid at position +76 is asparagine (N) in both B. amyloliquefaciens and B. lentus subtilisins. In the protease variants of the invention, however, the amino acid equivalent to +76 in Bacillus amyloliquefaciens subtilisin is substituted with aspartate (D). The abbreviations and one letter codes for all amino acids in the present invention conform to the Patentin User Manual (GenBank, Mountain View, CA) 1990, p. 101.

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"Equivalent residues" may also be defined by determining homology at the level of tertiary structure for a precursor protease whose tertiary structure has been determined by x-ray crystallography. Equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the precursor protease and Bacillus amyloliquefaciens subtilisin (N on N, CA on CA, C on C and O on O) are within 0.13nm and preferably 0.1nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the protease in question to the Bacillus amyloliquefaciens subtilisin. The best model is the crystallographic model giving the lowest R factor for experimental diffraction data at the highest resolution available.

$$R factor = \frac{\sum_{h} |Fo(h)| - |Fc(h)|}{\sum_{h} |Fo(h)|}$$

Equivalent residues which are functionally analogues to a specific residue of Bacillus amyloliquefaciens subtilisin are defined as those amino acids of the precursor protease which may adopt a conformation such that they either alter, modify or contribute to protein structure, substrate binding or catalysis in a manner defined and attributed to a specific residue of the Bacillus amyloliquefaciens subtilisin. Further, they are those residues of the precursor protease (for which a tertiary structure has been obtained by x-ray crystallography) which occupy an analogous position to the extent that, although the main chain atoms of the given residue may not satisfy the criteria of equivalence on the basis of occupying a homologous position, the atomic coordinates of at least two fo the side chain atoms of the residue lie with 0.13nm of the corresponding side chain atoms of Bacillus amyloliquefaciens subtilisin. The coordinates of the three dimensional structure of Bacillus amyloliquefaciens subtilisin are set forth in EPO Publication No. 0 251 446 (equivalent to US Patent 5,182,204, the disclosure of which is incorporated herein by reference) and can be used as outlined above to determine equivalent residues on the level of tertiary structure. Some of the residues identified for substitution, insertion or deletion are conserved residues whereas others are not. In the case of residues which are not conserved, the replacement of one or more amino acids is limited to substitutions which produce a variant which has an amino acid sequence that does not correspond to one found in nature. In the case of conserved residues, such replacements should not result in natural-occurring sequence. The protease variants of the present invention include the mature forms of protease variants, as well as the pro- and pre-pro-forms of such protease variants. The prepro-forms are the preferred construction since this facilitates the expression, secretion and maturation of the protease variants.

"Prosequence" refers to a sequence of amino acids bound to the N-terminal portion of the mature form of a protease which when removed results in the appearance of the "mature" form of the protease. Many proteolytic enzymes are found in nature as translational proenzyme products and, in the absence of post-translational processing, are expressed in this fashion. A preferred prosequence for producing protease variants is the putative prosequence of *Bacillus amyloliquefaciens* subtilisin, although other protease prosequences may be used.

A "signal sequence" or "presequence" refers to any sequence of amino acids bound to the N'terminal portion of a protease or to the N-terminal portion of a proprotease which may participate in the secretion of the mature or pro forms of the protease. This definition of signal sequence is a functional one, meant to include all those amino sequences encoded by the N-terminal portion of the protease gene which participate in the effectuation of the secretion of protease under native conditions. The present invention utilizes such sequences to effect the secretion of the protease variants as defined here. One possible signal sequence comprises the first seven amino acid residues of the signal sequence from Bacillus subtilis subtilisin fused to the remainder of the signal sequence of the subtilisin from Bacillus lentus_(ATCC 21536).

A "prepro" form of a protease variant consists of the mature form of the protease having a prosequence operably linked to the amino terminus of the protease and a "pre" or "signal" sequence operably linked to the amino terminus of the prosequence.

"Expression vector" refers to a DNA construct containing a DNA sequence which is operably linked to a suitable control sequence capable of effecting the expression of said DNA in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites and sequences which control termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently or the host genome, or may, in some instances, integrate into the

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genome itself. In the present specification, "plasmid" and "vector" are sometimes used interchangeably as the plasmid is the most commonly used form of vector at present. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which are, or become, known in the art.

The "host cells" used in the present invention generally are procaryotic or eucaryotic hosts which preferably have been manipulated by the methods disclosed in US Patent RE 34,606 to render them incapable of secreting enzymatically active endoprotease. A preferred host cell for expressing protease is the *Bacillus* strain BG2036 which is deficient in enzymatically active neutral protease and alkaline protease (subtilisin). The construction of strain BG2036 is described in detail in US Patent 5,264,366. Other host cells for expressing protease include *Bacillus subtilis* 168 (also described in US Patent RE 34,606 and US Patent 5,264,366, the disclosure of which are incorporated herein by reference), as well as any suitable *Bacillus* strain such as *B. licheniformis*, *B. lentus*, etc.).

Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the protease variants or expressing the desired protease variant. In the case of vectors which encode the pre- or prepro-form of the protease variant, such variants, when expressed, are typically secreted from the host cell in to the host cell medium.

"Operably linked, "when describing the relationship between two DNA regions, simply means that they are functionally related to each other. For example, a prosequence is operably linked to a peptide if it functions as a signal sequence, participating in the secretion of the mature form of the protein most probably involving cleavage of the signal sequence. A promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation.

The genes encoding the naturally-occurring precursor protease may be obtained in accord with the general methods known to those skilled in the art. The methods generally comprise synthesizing labeled probes having putative sequences encoding regions of the protease of interest, preparing genomic libraries from organisms expressing the protease, and screening the libraries for the gene of interest by hybridization to the probes. Positively hybridizing clones are then mapped and sequenced.

The cloned protease is then used to transform a host cell in order to express the protease. The protease gene is then ligated into a high copy number plasmid. This plasmid replicates in hosts in the sense that it contains the well-known elements necessary for plasmid replication: a promote operably linked to the gene in question (which may be supplied as the gene's own homologous promoter if it is recognized, i.e. transcribed by the host), a transcription termination and polyadenylation region (necessary for stability of the

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mRNA transcribed by the host from the protease gene in certain eucaryotic host cells) which is exogenous or is supplied by the endogenous terminator region of the protease gene and, desirably, a selection gene such as an antibiotic resistance gene that enables continuous cultural maintenance of plasmid-infected host cells by growth in antibioticcontaining media. High copy number plasmids also contain an origin of replication for the host, thereby enabling large numbers of plasmids to be generated in the cytoplasm without chromosomal limitation. However, it is within the scope herein to integrate multiple copies of the protease gene into host genome. This is facilitated by procaryotic and eucaryotic organisms which are particularly susceptible to homologous recombination. The gene can be a natural B. lentus gene. Alternatively, a synthetic gene encoding a naturallyoccurring or mutant precursor protease may be produced. In such an approach, the DNA and/or amino acid sequence of the precursor protease is determined. Multiple, overlapping synthetic single-stranded DNA fragments are thereafter synthesized, which upon hybridization and ligation produce a synthetic DNA enclding the precursor protease. An example of synthetic gene construction is set forth in Example 3 of US Patent 5,204,105, the disclosure of which is incorporated herein by reference.

Once the naturally-occurring or synthetic precursor protease gene has been cloned, a number of modifications are undertaken to enhance the use of the gene beyond synthesis of the naturally-occurring precursor protease. Such modifications include the production of recombinant proteases as disclosed in US Patent RE 34,606 and EPO Publication No. 0 251 446 and the production of protease variants described herein.

The following cassette mutagenesis method may be used to facilitate the construction of the proteases variants of the present invention, although other methods may be used. First, the naturally-occurring gene encoding the protease is obtained and sequenced in whole or in part. Then the sequence is scanned for a point at which it is desired to make a mutation (deletion, insertion or substitution) of one or more amino acids in the encoded enzyme. The sequences flanking this point are evaluated for the presence of restriction sites for replacing a short segment of the gene with an oligonucleotide pool which, when expressed will encode various mutants. Such restriction sites are preferably unique sites within the protease gene so as to facilitate the replacement of the gene segment. However, any convenient restriction site which is not overly redundant in the protease gene may be used, provided the gene fragments generated by restriction digestion can be reassembled in proper sequence. If restriction sites are not present at locations within a convenient distance from the selected point (from 10 to 15 nucleotides), such sites are generated by substituting nucleotides in the gene in such fashion that neither the reading frame nor the amino acids encoded are changed in the final construction. Mutation of the gene in order to change its sequence to conform to the desired sequence is accomplished by

M13 primer extension in accord with generally known methods. The task of locating suitable flanking regions and evaluating the needed changes to arrive at two convenient restriction site sequences is made routine by the redundancy of the genetic code, a restriction enzyme map of the gene and the large number of different restriction enzymes. Note that if a convenient flanking restriction site if available, the above method need be used only in connection with the flanking region which does not contain a site.

Once the naturally-occurring DNA or synthetic DNA is cloned, the restriction sites flanking the positions to be mutated are digested with the cognate restriction enzymes and a plurality of end termini-complementary oligonucleotide cassettes are ligated into the gene. The mutagenesis is simplified by this method because all of the oligonucleotides can be synthesized so as to have the same restriction sites, and no synthetic linkers are necessary to create the restriction sites. As used herein, proteolytic activity is defined as the rate of hydrolysis of peptide bonds per milligram of active enzyme. Many well known procedures exist for measuring proteolytic activity (K. M. Kalisz, "Microbial Proteinases," Advances in Biochemical Engineering/Biotechnology, A. Fiechter ed., 1988). In addition to or as an alternative to modified proteolytic activity, the variant enzymes of the present invention may have other modified properties such as K_m , k_{cat} , k_{cat} , k_m ratio and/or modified substrate specifically and/or modified pH activity profile. These enzymes can be tailored for the particular substrate which is anticipated to be present, for example, in the preparation of peptides or for hydrolytic processes such as laundry uses.

In one aspect of the invention, the objective is to secure a variant protease having altered proteolytic activity as compared to the precursor protease, since increasing such activity (numerically larger) enables the use of the enzyme to more efficiently act on a target substrate. Also of interest are variant enzymes having altered thermal stability and/or altered substrate specificity as compared to the precursor. In some instances, lower proteolytic activity may be desirable, for example a decrease in proteolytic activity would be useful where the synthetic activity of the proteases is desired (as for synthesizing peptides). One may wish to decrease this proteolytic activity, which is capable of destroying the product of such synthesis. Conversely, in some instances it may be desirable to increase the proteolytic activity of the variant enzyme versus its precursor. Additionally, increases or decreases (alteration) of the stability of the variant, whether alkaline or thermal stability, may be desirable. Increases or decreases in k_{cat} , K_m or K_{cat} / K_m are specific to the substrate used to determine these kinetic parameters.

In another aspect of the invention, it has been determined that substitutions at positions corresponding to 103 in combination with one or more of the following positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111,

114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of *Bacillus amyloliquefaciens* subtilisin are important in modulating overall stability and/or proteolytic activity of the enzyme.

In a further aspect of the invention, it has been determined that substitutions at one or more of the following positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin are also important in modulating overall stability and/or proteolytic activity of the enzyme.

These substitutions are preferably made in *Bacillus lentus* (recombinant or native-type) subtilisin, although the substitutions may be made in any *Bacillus* protease.

Based on the screening results obtained with the variant proteases, the noted mutations in *Bacillus amyloliquefaciens* subtilisin are important to the proteolytic activity, performance and/or stability of these enzymes and the cleaning or wash performance of such variant enzymes.

Methods and procedures for making the enzymes used in the detergent and cleaning compositions of the present invention are known and are disclosed in PCT Publication No. WO 95/10615.

The enzymes of the present invention have trypsin-like specificity. That is, the enzymes of the present invention hydrolyze proteins by preferentially cleaving the peptide bonds of charged amino acid residues, more specifically residues such as arginine and lysine, rather than preferentially cleaving the peptide bonds of hydrophobic amino acid residues, more specifically phenylalanine, tryptophan and tyrosine. Enzymes having the latter profile have a chymotrypsin-like specificity. Substrate specificity as discussed above is illustrated by the action of the enzyme on two synthetic substrates. Protease's having trypsin-like specificity hydrolyze the synthetic substrate bVGR-pNA preferentially over the synthetic substrate sucAAPF-pNA. Chymotrypsin-like protease enzymes, in contrast, hydrolyze the latter much faster than the former. For the purposes of the present invention the following procedure was employed to define the trypsin-like specificity of the protease enzymes of the present invention:

A fixed amount of a glycine buffer at a pH of 10 and a temperature of 25 °C is added to a standard 10 ml test tube. 0.5 ppm of the active enzyme to be tested is added to the test tube. Approximately, 1.25 mg of the synthetic substrate per mL of buffer solution is added to the test tube. The mixture is allowed to incubate for 15 minutes at 25 °C. Upon completion of the incubation period, an enzyme inhibitor, PMSF, is added to the mixture at

a level of 0.5 mg per mL of buffer solution. The absorbency or OD value of the mixture is read at a 410 nm wavelength. The absorbence then indicates the activity of the enzyme on the synthetic substrate. The greater the absorbence, the higher the level of activity against that substrate.

To then determine the specificity of an individual enzyme, the absorbence on the two synthetic substrate proteins may be converted into a specificity ratio. For the purposes of the present invention, the ratio is determined by the formula specificity of:

[activity on sAAPF-pNA]/[activity on bVGR-pNA]

An enzyme having a ratio of less than about 10, more preferably less than about 5 and most preferably less than about 2.5 may then be considered to demonstrate trypsin-like activity.

Such variants generally have at least one property which is different from the same property of the protease precursor from which the amino acid sequence of the variant is derived.

One aspect of the invention are compositions, such as detergent and cleaning compositions, for the treatment of textiles, dishware, tableware, kitchenware, cookware, and other hard surface substrates that include one or more of the variant proteases of the present invention. Protease-containing compositions can be used to treat for example: silk or wool, as well as other types of fabrics, as described in publications such as RD 216,034, EP 134,267, US 4,533,359, and EP 344,259; and dishware, tableware, kitchenware, cookware, and other hard surface substrates as described in publications such as in US 5,478,742, US 5,346,822, US 5,679,630, and US 5,677,272.

II. Amylase Variants - The amylase variants used in the present invention include, but are not limited to, the amylase enzymes described in WO 95/26397 and in WO 96/23873 (Novo). These enzymes are incorporated into cleaning compositions at a level of from about 0.0001%, preferably from about 0.00018%, more preferably from about 0.00024%, most preferably from about 0.05% to about 0.1%, preferably to about 0.060%, more preferably to about 0.048% by weight of the cleaning compositions of pure enzyme.

The amylase variants are preferably selected from the group consisting of $\,\alpha$ -amylase variants.

Suitable α -amylase variants for use in the present invention include, but are not limited to the following α -amylases:

(i) α -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas[®] α -amylase activity assay and/or;

- (ii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv) α -amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an α -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;
- (v) α -amylase according to (i-iv) wherein the α -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi) α -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii) α -amylase showing positive immunological cross-reactivity with antibodies raised against an α -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;

(viii) variant of a parent α -amylase, wherein the parent α -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an a-amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an α amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent \alpha-amylase has been deleted; and/or (B) at least one amino acid residue of said parent \(\alpha \)-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α-amylase; said variant having an α-amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or α -amylolytic activity at neutral to relatively high pH values; increased α -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for \alpha-amylase variant to the pH of the medium.

A polypeptide is considered to be X% homologous to the parent amylase if a comparison of the respective amino acid sequences, performed via algorithms, such as the one described by Lipman and Pearson in Science 227, 1985, p. 1435, reveals an identity of X%.

In the context of the present invention, the term "obtainable from" is intended not only to indicate an amylase produced by a Bacillus strain but also an amylase encoded by a DNA sequence isolated from such a Bacillus strain and produced in a host organism transformed with the DNA sequence.

III. Protease/Amylase Combination - Although any one or more of the protease variants described above can be combined with one or more of the amylase variants described above, in a highly preferred embodiment of the present invention, the protease variant comprises the substitution set: 101/103/104/159/232/236/245/248/252, and more highly preferred the substitution set: 101G/103A/104I/232V/236H/245R/248D/252K.

Although the protease variant and amylase variant can be present in the cleaning compositions in any ratio by ppm, a preferred ratio of protease variant(s) to amylase variant(s) by ppm in the cleaning compositions of the present invention are in the range of from about 1:20 to about 20:1, preferably from about 1:10 to about 10:1, more preferably from about 1:3 to about 3:1.

CLEANING COMPOSITIONS

The cleaning compositions of the present invention also comprise, in addition to one or more protease variants described hereinbefore, one or more cleaning adjunct materials, preferably compatible with the protease variant(s). The term "cleaning adjunct materials", as used herein, means any liquid, solid or gaseous material selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid; granule; powder; bar; paste; spray; tablet; gel; foam composition), which materials are also preferably compatible with the protease enzyme used in the composition. Granular compositions can also be in "compact" form and the liquid compositions can also be in a "concentrated" form.

The specific selection of cleaning adjunct materials are readily made by considering the surface, item or fabric to be cleaned, and the desired form of the composition for the cleaning conditions during use (e.g., through the wash detergent use). The term "compatible", as used herein, means the cleaning composition materials do not reduce the proteolytic activity of the protease enzyme to such an extent that the protease is not effective as desired during normal use situations. Examples of suitable cleaning adjunct materials include, but are not limited to, surfactants, builders, bleaches, bleach activators, bleach catalysts, other enzymes, enzyme stabilizing systems, chelants, optical brighteners, soil release polymers, dye transfer agents, dispersants, suds suppressors, dyes, perfumes, colorants, filler salts, hydrotropes, photoactivators, fluorescers, fabric conditioners, hydrolyzable surfactants, perservatives, anti-oxidants, anti-shrinkage agents, anti-wrinkle agents, germicides, fungicides, color speckles, silvercare, anti-tarnish and/or anti-corrosion agents, alkalinity sources, solubilizing agents, carriers, processing aids,

pigments and pH control agents as described in U.S. Patent Nos. 5,705,464, 5,710,115, 5,698,504, 5,695,679, 5,686,014 and 5,646,101. Specific cleaning composition materials are exemplified in detail hereinafter.

If the cleaning adjunct materials are not compatible with the protease variant(s) in the cleaning compositions, then suitable methods of keeping the cleaning adjunct materials and the protease variant(s) separate (not in contact with each other) until combination of the two components is appropriate can be used. Suitable methods can be any method known in the art, such as gelcaps, encapulation, tablets, physical separation, etc.

Preferably an effective amount of one or more protease variants described above are included in compositions useful for cleaning a variety of surfaces in need of proteinaceous stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form and including both granular and liquid automatic dishwashing); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations); and denture cleaning compositions, unlimited in form (e.g., liquid, tablet).

As used herein, "effective amount of protease variant" refers to the quantity of protease variant described hereinbefore necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like.

Preferably the cleaning compositions comprise from about 0.001%, preferably from about 0.001%, more preferably from about 0.01% by weight of the cleaning compositions of one or more protease variants of the present invention, to about 10%, preferably to about 1%, more preferably to about 0.1%. Also preferably the protease variant of the present invention is present in the compositions in an amount sufficient to provide a ratio of mg of active protease per 100 grams of composition to ppm theoretical Available O₂ ("AvO₂") from any peroxyacid in the wash liquor, referred to herein as the Enzyme to Bleach ratio (E/B ratio), ranging from about 1:1 to about 20:1. Several examples of various cleaning compositions wherein the protease variants of the present invention may be employed are discussed in further detail below. Also, the cleaning compositions may include from about 1% to about 99.9% by weight of the composition of the cleaning adjunct materials.

The cleaning compositions of the present invention may be in the form of "fabric cleaning compositions" or "non-fabric cleaning compositions."

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As used herein, "fabric cleaning compositions" include hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the soaking and/or pretreatment of stained fabrics.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing detergent compositions, oral cleaning compositions, denture cleaning compositions and personal cleansing compositions.

When the cleaning compositions of the present invention are formulated as compositions suitable for use in a laundry machine washing method, the compositions of the present invention preferably contain both a surfactant and a builder compound and additionally one or more cleaning adjunct materials preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. Laundry compositions can also contain softening agents, as additional cleaning adjunct

The compositions of the present invention can also be used as detergent additive products in solid or liquid form. Such additive products are intended to supplement or boost the performance of conventional detergent compositions and can be added at any stage of the cleaning process.

When formulated as compositions for use in manual dishwashing methods the compositions of the invention preferably contain a surfactant and preferably other cleaning adjunct materials selected from organic polymeric compounds, suds enhancing agents, group II metal ions, solvents, hydrotropes and additional enzymes.

If needed the density of the laundry detergent compositions herein ranges from 400 to 1200 g/litre, preferably 500 to 950 g/litre of composition measured at 20°C.

The "compact" form of the cleaning compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt; inorganic filler salts are conventional ingredients of detergent compositions in powder form; in conventional detergent compositions, the filler salts are present in substantial amounts, typically 17-35% by weight of the total composition. In the compact compositions, the filler salt is present in amounts not exceeding 15% of the total composition, preferably not exceeding 10%, most preferably not exceeding 5% by weight of the composition. The inorganic filler salts, such as meant in the present compositions are selected from the alkali and alkaline-earth-metal salts of sulfates and chlorides. A preferred filler salt is sodium sulfate.

Liquid cleaning compositions according to the present invention can also be in a "concentrated form", in such case, the liquid cleaning compositions according the present invention will contain a lower amount of water, compared to conventional liquid detergents. Typically the water content of the concentrated liquid cleaning composition is preferably less than 40%, more preferably less than 30%, most preferably less than 20% by weight of the cleaning composition.

Cleaning Adjunct Materials

Surfactant System - Detersive surfactants included in the fully-formulated cleaning compositions afforded by the present invention comprises at least 0.01%, preferably at least about 0.1%, more preferably at least about 0.5%, most preferably at least about 1% to about 60%, more preferably to about 35%, most preferably to about 30% by weight of cleaning composition depending upon the particular surfactants used and the desired effects.

The detersive surfactant can be nonionic, anionic, ampholytic, zwitterionic, cationic, semi-polar nonionic, and mixtures thereof, nonlimiting examples of which are disclosed in U.S. Patent Nos. 5,707,950 and 5,576,282. Preferred detergent and cleaning compositions comprise anionic detersive surfactants or mixtures of anionic surfactants with other surfactants, especially nonionic surfactants.

Nonlimiting examples of surfactants useful herein include the conventional C₁₁- C_{18} alkyl benzene sulfonates and primary, secondary and random alkyl sulfates, the C_{10} - C_{18} alkyl alkoxy sulfates, the C_{10} - C_{18} alkyl polyglycosides and their corresponding sulfated polyglycosides, C12-C18 alpha-sulfonated fatty acid esters, C12-C18 alkyl and alkyl phenol alkoxylates (especially ethoxylates and mixed ethoxy/propoxy), C_{12} - C_{18} betaines and sulfobetaines ("sultaines"), C₁₀-C₁₈ amine oxides, and the like. Other conventional useful surfactants are listed in standard texts.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated such that it promotes, or at least does not degrade, the stability of any enzyme in

Nonionic Surfactants - Polyethylene, polypropylene, and polybutylene oxide these compositions. condensates of alkyl phenols are suitable for use as the nonionic surfactant of the surfactant systems of the present invention, with the polyethylene oxide condensates being preferred. Commercially available nonionic surfactants of this type include IgepalTM CO-630, marketed by the GAF Corporation; and TritonTM X-45, X-114, X-100 and X-102, all marketed by the Rohm & Haas Company. These surfactants are commonly referred to as alkylphenol alkoxylates (e.g., alkyl phenol ethoxylates).

The condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethylene oxide are suitable for use as the nonionic surfactant of the nonionic surfactant systems of the present invention. Examples of commercially available nonionic surfactants of this type include TergitolTM 15-S-9 (the condensation product of C₁₁-C₁₅ linear alcohol with 9 moles ethylene oxide), TergitolTM 24-L-6 NMW (the condensation product of C₁₂-C₁₄ primary alcohol with 6 moles ethylene oxide with a narrow molecular weight distribution), both marketed by Union Carbide Corporation; NeodolTM 45-9 (the condensation product of C₁₄-C₁₅ linear alcohol with 9 moles of ethylene oxide), NeodolTM 23-3 (the condensation product of C₁₂-C₁₃ linear alcohol with 3.0 moles of ethylene oxide), NeodolTM 45-7 (the condensation product of C₁₄-C₁₅ linear alcohol with 7 moles of ethylene oxide), NeodolTM 45-5 (the condensation product of C₁₄-C₁₅ linear alcohol with 5 moles of ethylene oxide) marketed by Shell Chemical Company, KyroTM EOB (the condensation product of C₁₃-C₁₅ alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company, and Genapol LA O3O or O5O (the condensation product of C₁₂-C₁₄ alcohol with 3 or 5 moles of ethylene oxide) marketed by Hoechst. Preferred range of HLB in these products is from 8-11 and most preferred from 8-10

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Also useful as the nonionic surfactant of the surfactant systems of the present invention are the alkylpolysaccharides disclosed in U.S. Patent No. 4,565,647.

Preferred alkylpolyglycosides have the formula: $R^2O(C_nH_{2n}O)_t(glycosyl)_x$ wherein R^2 is selected from the group consisting of alkyl, alkylphenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures thereof in which the alkyl groups contain from about 10 to about 18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, preferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 2.7.

The condensation products of ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol are also suitable for use as the additional nonionic surfactant systems of the present invention. Examples of compounds of this type include certain of the commercially-available Plurafac TM LF404 and Pluronic TM surfactants, marketed by BASF.

Also suitable for use as the nonionic surfactant of the nonionic surfactant system of the present invention, are the condensation products of ethylene oxide with the product resulting from the reaction of propylene oxide and ethylenediamine. Examples of this type of nonionic surfactant include certain of the commercially available Tetronic TM compounds, marketed by BASF.

Preferred for use as the nonionic surfactant of the surfactant systems of the present invention are polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethylene oxide, alkylpolysaccharides, and mixtures thereof. Most preferred are C₈-C₁₄ alkyl phenol

ethoxylates having from 3 to 15 ethoxy groups and C_{8} - C_{18} alcohol ethoxylates (preferably C_{10} avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula: R^2 - C(O) - $N(R^1)$ - Z wherein R^1 is H, or R^1 is C_{1-4} hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof, R^2 is C_{5-31} hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R^1 is methyl, R^2 is a straight C_{11-15} alkyl or C_{16-18} alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

Anionic Surfactants - Suitable anionic surfactants to be used are linear alkyl benzene sulfonate, alkyl ester sulfonate surfactants including linear esters of C₈-C₂₀ carboxylic acids (i.e., fatty acids) which are sulfonated with gaseous SO₃ according to "The Journal of the American Oil Chemists Society", 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm oil, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprise alkyl ester sulfonate surfactants of the structural formula:

wherein R^3 is a C_8 - C_{20} hydrocarbyl, preferably an alkyl, or combination thereof, R^4 is a C_1 - C_6 hydrocarbyl, preferably an alkyl, or combination thereof, and M is a cation which forms a water soluble salt with the alkyl ester sulfonate. Suitable salt-forming cations include metals such as sodium, potassium, and lithium, and substituted or unsubstituted ammonium cations, such as monoethanolamine, diethanolamine, and triethanolamine. Preferably, R^3 is C_{10} - C_{16} alkyl, and R^4 is methyl, ethyl or isopropyl. Especially preferred are the methyl ester sulfonates wherein R^3 is C_{10} - C_{16} alkyl.

Other suitable anionic surfactants include the alkyl sulfate surfactants which are water soluble salts or acids of the formula ROSO₃M wherein R preferably is a C_{10} - C_{24} hydrocarbyl, preferably an alkyl or hydroxyalkyl having a C_{10} - C_{20} alkyl component, more preferably a C_{12} - C_{18} alkyl or hydroxyalkyl, and M is H or a cation. Typically, alkyl chains of C_{12} - C_{16} are preferred for lower wash temperatures (e.g. below about 50°C) and C_{16} -18 alkyl chains are preferred for higher wash temperatures (e.g. above about 50°C).

Other anionic surfactants useful for detersive purposes include salts of soap, C₈-C₂₂ primary of secondary alkanesulfonates, C₈-C₂₄ olefinsulfonates, sulfonated

polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in British patent specification No. 1,082,179, Cg-C24 alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide); alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinates (especially saturated and unsaturated C12-C18 monoesters) and diesters of sulfosuccinates (especially saturated and unsaturated C6-C12 diesters), acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, and alkyl polyethoxy carboxylates such as those of the formula RO(CH2CH2O)k-CH2COO-M+ wherein R is a C8-C22 alkyl, k is an integer from 1 to 10, and M is a soluble salt-forming cation. Resin acids and hydrogenated resin acids are also suitable, such as rosin, hydrogenated rosin, and resin acids and hydrogenated resin acids present in or derived from tall oil.

Further examples are described in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perry and Berch). A variety of such surfactants are also generally disclosed in U.S. Patent 3,929,678, issued December 30, 1975 to Laughlin, et al. at Column 23, line 58 through Column 29, line 23 (herein incorporated by reference).

Highly preferred anionic surfactants include alkyl alkoxylated sulfate surfactants hereof are water soluble salts or acids of the formula RO(A)_mSO3M wherein R is an unsubstituted C10-C24 alkyl or hydroxyalkyl group having a C10-C24 alkyl component, preferably a C_{12} - C_{20} alkyl or hydroxyalkyl, more preferably C_{12} - C_{18} alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like. Exemplary surfactants are C₁₂-C₁₈ alkyl polyethoxylate (1.0) sulfate (C_{12} - $C_{18}E(1.0)M$), C_{12} - C_{18} alkyl polyethoxylate (2.25) sulfate (C_{12} - $C_{18}E(2.25)M$), C_{12} - C_{18} alkyl polyethoxylate (3.0) sulfate (C_{12} - $C_{18}E(3.0)M$), and C_{12} - C_{18} alkyl polyethoxylate (4.0) sulfate (C_{12} - $C_{18}E(4.0)M$), wherein M is conveniently selected from sodium and potassium.

When included therein, the cleaning compositions of the present invention typically comprise from about 1%, preferably from about 3% to about 40%, preferably about 20% by weight of such anionic surfactants.

Cationic Surfactants - Cationic detersive surfactants suitable for use in the cleaning compositions of the present invention are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyltrimethylammonium halogenides, and those surfactants having the formula: $[R^2(OR^3)_y][R^4(OR^3)_y]_2R^5N+X-\text{ wherein }R^2\text{ is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each <math>R^3$ is selected from the group consisting of -CH2CH2-, -CH2CH(CH3)-, -CH2CH(CH2OH)-, -CH2CH2CH2-, and mixtures thereof; each R^4 is selected from the group consisting of C_1 - C_4 alkyl, C_1 - C_4 hydroxyalkyl, benzyl ring structures formed by joining the two R^4 groups, -CH2CHOH-CHOHCOR6CHOHCH2OH wherein R^6 is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when y is not 0; R^5 is the same as R^4 or is an alkyl chain wherein the total number of carbon atoms of R^2 plus R^5 is not more than about 18; each y is from 0 to about 10 and the sum of the y values is from 0 to about 15; and X is any compatible anion.

Highly preferred cationic surfactants are the water-soluble quaternary ammonium compounds useful in the present composition having the formula (i): $R_1R_2R_3R_4N^+X^-$ wherein R_1 is C_8 - C_{16} alkyl, each of R_2 , R_3 and R_4 is independently C_1 - C_4 alkyl, C_1 - C_4 hydroxy alkyl, benzyl, and - $(C_2H_{40})_xH$ where x has a value from 2 to 5, and X is an anion. Not more than one of R_2 , R_3 or R_4 should be benzyl. The preferred alkyl chain length for R_1 is C_{12} - C_{15} particularly where the alkyl group is a mixture of chain lengths derived from coconut or palm kernel fat or is derived synthetically by olefin build up or OXO alcohols synthesis. Preferred groups for R_2R_3 and R_4 are methyl and hydroxyethyl groups and the anion X may be selected from halide, methosulfate, acetate and phosphate ions.

Examples of suitable quaternary ammonium compounds of formulae (i) for use herein are include, but are not limited to: coconut trimethyl ammonium chloride or bromide; coconut methyl dihydroxyethyl ammonium chloride or bromide; decyl triethyl ammonium chloride; decyl dimethyl hydroxyethyl ammonium chloride or bromide; C₁₂₋₁₅ dimethyl hydroxyethyl ammonium chloride or bromide; myristyl trimethyl ammonium methyl hydroxyethyl ammonium chloride or bromide; myristyl trimethyl ammonium methyl sulphate; lauryl dimethyl benzyl ammonium chloride or bromide; lauryl dimethyl (ethenoxy)₄ ammonium chloride or bromide; choline esters (compounds of formula (i) wherein R₁ is CH₂-CH₂-O-C-C₁₂₋₁₄ alkyl and R₂R₃R₄ are methyl); and di-alkyl imidazolines [(i)].

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Other cationic surfactants useful herein are also described in U.S. Patent 4,228,044, Cambre, issued October 14, 1980 and in European Patent Application EP 000,224.

When included therein, the cleaning compositions of the present invention typically comprise from about 0.2%, preferably from about 1% to about 25%, preferably to about 8% by weight of such cationic surfactants.

Ampholytic Surfactants - Ampholytic surfactants, examples of which are described in U.S. Patent No. 3,929,678, are also suitable for use in the cleaning compositions of the present invention.

When included therein, the cleaning compositions of the present invention typically comprise from about 0.2%, preferably from about 1% to about 15%, preferably to about 10% by weight of such ampholytic surfactants.

Zwitterionic Surfactants - Zwitterionic surfactants, examples of which are described in U.S. Patent No. 3,929,678, are also suitable for use in cleaning compositions.

When included therein, the cleaning compositions of the present invention typically comprise from about 0.2%, preferably from about 1% to about 15%, preferably to about 10% by weight of such zwitterionic surfactants.

<u>Semi-polar Nonionic Surfactants</u> - Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides having the formula:

 \uparrow $R^{3}(OR^{4})_{x}N(R^{5})_{2}$

wherein R³ is an alkyl, hydroxyalkyl, or alkyl phenyl group or mixtures thereof containing from about 8 to about 22 carbon atoms; R⁴ is an alkylene or hydroxyalkylene group containing from about 2 to about 3 carbon atoms or mixtures thereof; x is from 0 to about 3; and each R⁵ is an alkyl or hydroxyalkyl group containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups (the R⁵ groups can be attached to each other, e.g., through an oxygen or nitrogen atom, to form a ring structure); water-soluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

The amine oxide surfactants in particular include C_{10} - C_{18} alkyl dimethyl amine oxides and C_{8} - C_{12} alkoxy ethyl dihydroxy ethyl amine oxides.

When included therein, the cleaning compositions of the present invention typically comprise from about 0.2%, preferably from about 1% to about 15%, preferably to about 10% by weight of such semi-polar nonionic surfactants.

Cosurfactants - The cleaning compositions of the present invention may further comprise a cosurfactant selected from the group of primary or tertiary amines. Suitable primary amines for use herein include amines according to the formula R₁NH₂ wherein R₁ is a C₆-C₁₂, preferably C₆-C₁₀ alkyl chain or R₄X(CH_{2)n}, X is -O-,-C(O)NH- or -NH-, R₄ is a C₆-C₁₂ alkyl chain n is between 1 to 5, preferably 3. R₁ alkyl chains may be straight or branched and may be interrupted with up to 12, preferably less than 5 ethylene oxide moieties.

Preferred amines according to the formula herein above are n-alkyl amines. Suitable amines for use herein may be selected from 1-hexylamine, 1-octylamine, 1-decylamine and laurylamine. Other preferred primary amines include C8-C10 oxypropylamine, octyloxypropylamine, 2-ethylhexyl-oxypropylamine, lauryl amido propylamine and amido propylamine. The most preferred amines for use in the compositions herein are 1-hexylamine, 1-octylamine, 1-decylamine, 1-dodecylamine. Especially desirable are n-dodecyldimethylamine and bishydroxyethylcoconutalkylamine and oleylamine 7 times ethoxylated, lauryl amido propylamine and cocoamido propylamine.

LFNIs - Particularly preferred surfactants in the automatic dishwashing compositions (ADD) of the present invention are low foaming nonionic surfactants (LFNI) which are described in U.S. Patent Nos. 5,705,464 and 5,710,115. LFNI may be present in amounts from 0.01% to about 10% by weight, preferably from about 0.1% to about 10%, and most preferably from about 0.25% to about 4%. LFNIs are most typically used in ADDs on account of the improved water-sheeting action (especially from glass) which they confer to the ADD product. They also encompass non-silicone, nonphosphate polymeric materials further illustrated hereinafter which are known to defoam food soils encountered in automatic dishwashing.

Preferred LFNIs include nonionic alkoxylated surfactants, especially ethoxylates derived from primary alcohols, and blends thereof with more sophisticated surfactants, such as the polyoxypropylene/polyoxyethylene/polyoxypropylene (PO/EO/PO) reverse block polymers as described in U.S. Patent Nos. 5,705,464 and 5,710,115.

LFNIs which may also be used include those POLY-TERGENT® SLF-18 nonionic surfactants from Olin Corp., and any biodegradable LFNI having the melting point properties discussed hereinabove.

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These and other nonionic surfactants are well known in the art, being described in more detail in Kirk Othmer's Encyclopedia of Chemical Technology, 3rd Ed., Vol. 22, pp. 360-379, "Surfactants and Detersive Systems", incorporated by reference herein.

Bleaching System - The cleaning compositions of the present invention preferably comprise a bleaching system. Bleaching systems typically comprise a "bleaching agent" (source of hydrogen peroxide) and an "initiator" or "catalyst". When present, bleaching agents will typically be at levels of from about 1%, preferably from about 5% to about 30%, preferably to about 20% by weight of the composition. If present, the amount of bleach activator will typically be from about 0.1%, preferably from about 0.5% to about 60%, preferably to about 40% by weight, of the bleaching composition comprising the bleaching agent-plus-bleach activator.

Bleaching Agents - Hydrogen peroxide sources are described in detail in the herein incorporated Kirk Othmer's Encyclopedia of Chemical Technology, 4th Ed (1992, John Wiley & Sons), Vol. 4, pp. 271-300 "Bleaching Agents (Survey)", and include the various forms of sodium perborate and sodium percarbonate, including various coated and modified forms.

The preferred source of hydrogen peroxide used herein can be any convenient source, including hydrogen peroxide itself. For example, perborate, e.g., sodium perborate (any hydrate but preferably the mono- or tetra-hydrate), sodium carbonate peroxyhydrate or equivalent percarbonate salts, sodium pyrophosphate peroxyhydrate, urea peroxyhydrate, or sodium peroxide can be used herein. Also useful are sources of available oxygen such as persulfate bleach (e.g., OXONE, manufactured by DuPont). Sodium perborate monohydrate and sodium percarbonate are particularly preferred. Mixtures of any convenient hydrogen peroxide sources can also be used.

A preferred percarbonate bleach comprises dry particles having an average particle size in the range from about 500 micrometers to about 1,000 micrometers, not more than about 10% by weight of said particles being smaller than about 200 micrometers and not more than about 10% by weight of said particles being larger than about 1,250 micrometers. Optionally, the percarbonate can be coated with a silicate, borate or water-soluble surfactants. Percarbonate is available from various commercial sources such as FMC, Solvay and Tokai Denka.

Compositions of the present invention may also comprise as the bleaching agent a chlorine-type bleaching material. Such agents are well known in the art, and include for example sodium dichloroisocyanurate ("NaDCC"). However, chlorine-type bleaches are less preferred for compositions which comprise enzymes.

(a) Bleach Activators - Preferably, the peroxygen bleach component in the composition is formulated with an activator (peracid precursor). The activator is present at

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levels of from about 0.01%, preferably from about 0.5%, more preferably from about 1% to about 15%, preferably to about 10%, more preferably to about 8%, by weight of the composition. Preferred activators are selected from the group consisting of tetraacetyl ethylene diamine (TAED), benzoylcaprolactam (BzCL), 4-nitrobenzoylcaprolactam, 3-chlorobenzoylcaprolactam, benzoyloxybenzenesulphonate (BOBS), nonanoyloxybenzenesulphonate (NOBS), phenyl benzoate (PhBz), decanoyloxybenzenesulphonate (C₁₀-OBS), benzoylvalerolactam (BZVL), octanoyloxybenzenesulphonate (C₈-OBS), perhydrolyzable esters and mixtures thereof, most preferably benzoylcaprolactam and benzoylvalerolactam. Particularly preferred bleach activators in the pH range from about 8 to about 9.5 are those selected having an OBS or VL leaving group.

Preferred hydrophobic bleach activators include, but are not limited to, nonanoyloxybenzenesulphonate (NOBS), 4-[N-(nonaoyl) amino hexanoyloxy]-benzene sulfonate sodium salt (NACA-OBS) an example of which is described in U.S. Patent No. 5,523,434, dodecanoyloxybenzenesulphonate (LOBS or C_{12} -OBS), 10-undecenoyloxybenzenesulfonate (UDOBS or C_{11} -OBS with unsaturation in the 10 position), and decanoyloxybenzoic acid (DOBA).

Preferred bleach activators are those described in U.S. 5,698,504 Christie et al., issued December 16, 1997; U.S. 5,695,679 Christie et al. issued December 9, 1997; U.S. 5,686,401 Willey et al., issued November 11, 1997; U.S. 5,686,014 Hartshorn et al., issued November 11, 1997; U.S. 5,405,412 Willey et al., issued April 11, 1995; U.S. 5,405,413 Willey et al., issued April 11, 1995; U.S. 5,130,045 Mitchel et al., issued July 14, 1992; and U.S. 4,412,934 Chung et al., issued November 1, 1983, and copending patent applications U. S. Serial Nos. 08/709,072, 08/064,564, all of which are incorporated herein by reference.

The mole ratio of peroxygen bleaching compound (as AvO) to bleach activator in the present invention generally ranges from at least 1:1, preferably from about 20:1, more preferably from about 10:1 to about 1:1, preferably to about 3:1.

Quaternary substituted bleach activators may also be included. The present cleaning compositions preferably comprise a quaternary substituted bleach activator (QSBA) or a quaternary substituted peracid (QSP); more preferably, the former. Preferred QSBA structures are further described in U.S. 5,686,015 Willey et al., issued November 11, 1997; U.S. 5,654,421 Taylor et al., issued August 5, 1997; U.S. 5,460,747 Gosselink et al., issued October 24, 1995; U.S. 5,584,888 Miracle et al., issued December 17, 1996; and U.S. 5,578,136 Taylor et al., issued November 26, 1996; all of which are incorporated herein by reference.

Highly preferred bleach activators useful herein are amide-substituted as described in U.S. 5,698,504, U.S. 5,695,679, and U.S. 5,686,014 each of which are cited herein above. Preferred examples of such bleach activators include: (6-octanamidocaproyl)

oxybenzenesulfonate, (6-nonanamidocaproyl)oxybenzenesulfonate, (6-decanamidocaproyl)oxybenzenesulfonate and mixtures thereof.

Other useful activators, disclosed in U.S. 5,698,504, U.S. 5,695,679, U.S. 5,686,014 each of which is cited herein above and U.S. 4,966,723Hodge et al., issued October 30, 1990, include benzoxazin-type activators, such as a C_6H_4 ring to which is fused in the 1,2-positions a moiety --C(O)OC(R¹)=N-.

Depending on the activator and precise application, good bleaching results can be obtained from bleaching systems having with in-use pH of from about 6 to about 13, preferably from about 9.0 to about 10.5. Typically, for example, activators with electron-withdrawing moieties are used for near-neutral or sub-neutral pH ranges. Alkalis and buffering agents can be used to secure such pH.

Acyl lactam activators, as described in U.S. 5,698,504, U.S. 5,695,679 and U.S. 5,686,014, each of which is cited herein above, are very useful herein, especially the acyl caprolactams (see for example WO 94-28102 A) and acyl valerolactams (see U.S. 5,503,639 Willey et al., issued April 2, 1996 incorporated herein by reference).

- (b) Organic Peroxides, especially Diacyl Peroxides These are extensively illustrated in Kirk Othmer, Encyclopedia of Chemical Technology, Vol. 17, John Wiley and Sons, 1982 at pages 27-90 and especially at pages 63-72, all incorporated herein by reference. If a diacyl peroxide is used, it will preferably be one which exerts minimal adverse impact on spotting/filming.
- (c) Metal-containing Bleach Catalysts The present invention compositions and methods may utilize metal-containing bleach catalysts that are effective for use in bleaching compositions. Preferred are manganese and cobalt-containing bleach catalysts.

One type of metal-containing bleach catalyst is a catalyst system comprising a transition metal cation of defined bleach catalytic activity, such as copper, iron, titanium, ruthenium tungsten, molybdenum, or manganese cations, an auxiliary metal cation having little or no bleach catalytic activity, such as zinc or aluminum cations, and a sequestrate having defined stability constants for the catalytic and auxiliary metal cations, particularly ethylenediaminetetraacetic acid, ethylenediaminetetra (methylenephosphonic acid) and water-soluble salts thereof. Such catalysts are disclosed in U.S. 4,430,243 Bragg, issued February 2, 1982.

Manganese Metal Complexes - If desired, the compositions herein can be catalyzed by means of a manganese compound. Such compounds and levels of use are well known in the art and include, for example, the manganese-based catalysts disclosed in U.S. Patent Nos. 5,576,282; 5,246,621; 5,244,594; 5,194,416; and 5,114,606; and European Pat. App. Pub. Nos. 549,271 A1, 549,272 A1, 544,440 A2, and 544,490 A1; Preferred examples of these catalysts include Mn^{IV}₂(u-O)₃(1,4,7-trimethyl-1,4,7-triazacyclononane)₂(PF₆)₂,

MnIII₂(u-O)₁(u-OAc)₂(1,4,7-trimethyl-1,4,7-triazacyclononane)₂(ClO₄)₂, MnIV₄(u-O)₆(1,4,7-triazacyclononane)₄(ClO₄)₄, MnIII_{Mn}IV₄(u-O)₁(u-OAc)₂-(1,4,7-trimethyl-1,4,7-triazacyclononane)₂(ClO₄)₃, MnIV₄(1,4,7-trimethyl-1,4,7-triazacyclononane)₂(OCH₃)₃(PF₆), and mixtures thereof. Other metal-based bleach catalysts include those disclosed in U.S. Patent Nos. 4,430,243 and U.S. 5,114,611. The use of manganese with various complex ligands to enhance bleaching is also reported in the following: U.S. Patent Nos. 4,728,455; 5,284,944; 5,246,612; 5,256,779; 5,280,117; 5,274,147; 5,153,161; and 5,227,084.

Cobalt Metal Complexes - Cobalt bleach catalysts useful herein are known, and are described, for example, in U.S. Patent Nos. 5,597,936; 5,595,967; and 5,703,030; and M. L. Tobe, "Base Hydrolysis of Transition-Metal Complexes", Adv. Inorg. Bioinorg. Mech., (1983), 2, pages 1-94. The most preferred cobalt catalyst useful herein are cobalt pentaamine acetate salts having the formula [Co(NH₃)₅OAc] T_y, wherein "OAc" represents an acetate moiety and "T_y" is an anion, and especially cobalt pentaamine acetate chloride, [Co(NH₃)₅OAc]Cl₂; as well as [Co(NH₃)₅OAc](OAc)₂; [Co(NH₃)₅OAc](PF₆)₂; [Co(NH₃)₅OAc](SO₄); [Co(NH₃)₅OAc](BF₄)₂; and [Co(NH₃)₅OAc](NO₃)₂ (herein "PAC").

These cobalt catalysts are readily prepared by known procedures, such as taught for example in U.S. Patent Nos. 5,597,936; 5,595,967; and 5,703,030; in the Tobe article and the references cited therein; and in U.S. Patent 4,810,410; J. Chem. Ed. (1989), 66 (12), 1043-45; The Synthesis and Characterization of Inorganic Compounds, W.L. Jolly (Prentice-Hall; 1970), pp. 461-3; Inorg. Chem., 18, 1497-1502 (1979); Inorg. Chem., 21, 2881-2885 (1982); Inorg. Chem., 18, 2023-2025 (1979); Inorg. Synthesis, 173-176 (1960); and Journal of Physical Chemistry, 56, 22-25 (1952).

Transition Metal Complexes of Macropolycyclic Rigid Ligands - Compositions herein may also suitably include as bleach catalyst a transition metal complex of a macropolycyclic rigid ligand. The phrase "macropolycyclic rigid ligand" is sometimes abbreviated as "MRL" in discussion below. The amount used is a catalytically effective amount, suitably about 1 ppb or more, for example up to about 99.9%, more typically about 0.001 ppm or more, preferably from about 0.05 ppm to about 500 ppm (wherein "ppb" denotes parts per billion by weight and "ppm" denotes parts per million by weight).

Suitable transition metals e.g., Mn are illustrated hereinafter. "Macropolycyclic" means a MRL is both a macrocycle and is polycyclic. "Polycyclic" means at least bicyclic. The term "rigid" as used herein herein includes "having a superstructure" and "cross-bridged". "Rigid" has been defined as the constrained converse of flexibility: see D.H. Busch., Chemical Reviews., (1993), 93, 847-860, incorporated by reference. More particularly, "rigid" as used herein means that the MRL must be determinably more rigid

than a macrocycle ("parent macrocycle") which is otherwise identical (having the same ring size and type and number of atoms in the main ring) but lacking a superstructure (especially linking moieties or, preferably cross-bridging moieties) found in the MRL's. In determining the comparative rigidity of macrocycles with and without superstructures, the practitioner will use the free form (not the metal-bound form) of the macrocycles. Rigidity is well-known to be useful in comparing macrocycles; suitable tools for determining, measuring or comparing rigidity include computational methods (see, for example, Zimmer, Chemical Reviews, (1995), 95(38), 2629-2648 or Hancock et al., Inorganica Chimica Acta, (1989), 164, 73-84.

Preferred MRL's herein are a special type of ultra-rigid ligand which is cross-bridged. A "cross-bridge" is nonlimitingly illustrated in 1.11 hereinbelow. In 1.11, the cross-bridge is a -CH₂CH₂- moiety. It bridges N¹ and N⁸ in the illustrative structure. By comparison, a "same-side" bridge, for example if one were to be introduced across N¹ and N¹² in 1.11, would not be sufficient to constitute a "cross-bridge" and accordingly would not be preferred.

Suitable metals in the rigid ligand complexes include Mn(II), Mn(III), Mn(IV), Mn(V), Fe(II), Fe(III), Fe(IV), Co(I), Co(II), Co(III), Ni(I), Ni(II), Ni(III), Cu(II), Cu(III), Cu(III), Cr(III), Cr(III), Cr(IV), Cr(V), Cr(VI), V(III), V(IV), V(V), Mo(IV), Mo(V), Mo(VI), W(VI), W(VI), Pd(II), Ru(III), Ru(III), and Ru(IV). Preferred transition-metals in the instant transition-metal bleach catalyst include manganese, iron and chromium.

More generally, the MRL's (and the corresponding transition-metal catalysts) herein suitably comprise:

- (a) at least one macrocycle main ring comprising four or more heteroatoms; and
- (b) a covalently connected non-metal superstructure capable of increasing the rigidity of the macrocycle, preferably selected from
- (i) a bridging superstructure, such as a linking moiety;
- (ii) a cross-bridging superstructure, such as a cross-bridging linking moiety; and
- (iii) combinations thereof.

The term "superstructure" is used herein as defined in the literature by Busch et al., see, for example, articles by Busch in "Chemical Reviews".

Preferred superstructures herein not only enhance the rigidity of the parent macrocycle, but also favor folding of the macrocycle so that it co-ordinates to a metal in a cleft. Suitable superstructures can be remarkably simple, for example a linking moiety such as any of those illustrated in Fig. 1 and Fig. 2 below, can be used.



Fig. 1

wherein n is an integer, for example from 2 to 8, preferably less than 6, typically 2 to 4, or

$$(CH_2)_{m}$$
 $(CH_2)_{r}$

Fig. 2

wherein m and n are integers from about 1 to 8, more preferably from 1 to 3; Z is N or CH; and T is a compatible substituent, for example H, alkyl, trialkylammonium, halogen, nitro, sulfonate, or the like. The aromatic ring in 1.10 can be replaced by a saturated ring, in which the atom in Z connecting into the ring can contain N, O, S or C.

Suitable MRL's are further nonlimitingly illustrated by the following compound:

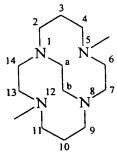


Fig. 3

This is a MRL in accordance with the invention which is a highly preferred, cross-bridged, methyl-substituted (all nitrogen atoms tertiary) derivative of cyclam. Formally, this ligand is named 5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane using the extended von Baeyer system. See "A Guide to IUPAC Nomenclature of Organic Compounds: Recommendations 1993", R. Panico, W.H. Powell and J-C Richer (Eds.), Blackwell Scientific Publications, Boston, 1993; see especially section R-2.4.2.1.

Transition-metal bleach catalysts of Macrocyclic Rigid Ligands which are suitable for use in the invention compositions can in general include known compounds where they conform with the definition herein, as well as, more preferably, any of a large number of novel compounds expressly designed for the present laundry or cleaning uses, and non-limitingly illustrated by any of the following:

Dichloro-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II)

Diaquo-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II)

Hexafluorophosphate

Aquo-hydroxy-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(III) Hexafluorophosphate

Diaquo-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II) Tetrafluoroborate

Dichloro-5,12-dimethyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(III) Hexafluorophosphate

Dichloro-5,12-di-n-butyl-1,5,8,12-tetraaza bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5,12-dibenzyl-1,5,8,12-tetraazabicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-butyl-12-methyl-1,5,8,12-tetraaza- bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-octyl-12-methyl-1,5,8,12-tetraaza- bicyclo[6.6.2]hexadecane Manganese(II)

Dichloro-5-n-butyl-12-methyl-1,5,8,12-tetraaza- bicyclo[6.6.2]hexadecane Manganese(II).

As a practical matter, and not by way of limitation, the compositions and cleaning processes herein can be adjusted to provide on the order of at least one part per hundred million of the active bleach catalyst species in the aqueous washing medium, and will preferably provide from about 0.01 ppm to about 25 ppm, more preferably from about 0.05 ppm to about 10 ppm, and most preferably from about 0.1 ppm to about 5 ppm, of the bleach catalyst species in the wash liquor. In order to obtain such levels in the wash liquor of an automatic washing process, typical compositions herein will comprise from about 0.0005% to about 0.2%, more preferably from about 0.004% to about 0.08%, of bleach catalyst, especially manganese or cobalt catalysts, by weight of the bleaching compositions.

(d) Other Bleach Catalysts - The compositions herein may comprise one or more other bleach catalysts. Preferred bleach catalysts are zwitterionic bleach catalysts, which are described in U.S. Patent No. 5,576,282 (especially 3-(3,4-dihydroisoquinolinium) propane sulfonate. Other bleach catalysts include cationic bleach catalysts are described in U.S. Patent Nos. 5,360,569, 5,442,066, 5,478,357, 5,370,826, 5,482,515, 5,550,256, and WO 95/13351, WO 95/13352, and WO 95/13353.

Also suitable as bleaching agents are preformed peracids, such as phthalimido-peroxy-caproic acid ("PAP"). See for example U.S. Patent Nos. 5,487,818, 5,310,934, 5,246,620, 5,279,757 and 5,132,431.

Optional Detersive Enzymes - The detergent and cleaning compositions herein may also optionally contain one or more types of detergent enzymes. Such enzymes can include other proteases, amylases, cellulases and lipases. Such materials are known in the art and are commercially available under such trademarks as. They may be incorporated into the non-aqueous liquid detergent compositions herein in the form of suspensions, "marumes" or "prills". Another suitable type of enzyme comprises those in the form of slurries of enzymes in nonionic surfactants, e.g., the enzymes marketed by Novo Nordisk under the tradename "SL" or the microencapsulated enzymes marketed by Novo Nordisk under the tradename "LDP." Suitable enzymes and levels of use are described in U.S. Pat. No. 5,576,282, 5,705,464 and 5,710,115.

Enzymes added to the compositions herein in the form of conventional enzyme prills are especially preferred for use herein. Such prills will generally range in size from about 100 to 1,000 microns, more preferably from about 200 to 800 microns and will be suspended throughout the non-aqueous liquid phase of the composition. Prills in the compositions of the present invention have been found, in comparison with other enzyme forms, to exhibit especially desirable enzyme stability in terms of retention of enzymatic activity over time. Thus, compositions which utilize enzyme prills need not contain conventional enzyme stabilizing such as must frequently be used when enzymes are incorporated into aqueous liquid detergents.

However, enzymes added to the compositions herein may be in the form of granulates, preferably T-granulates.

"Detersive enzyme", as used herein, means any enzyme having a cleaning, stain removing or otherwise beneficial effect in a laundry, hard surface cleaning or personal care detergent composition. Preferred detersive enzymes are hydrolases such as proteases, amylases and lipases. Preferred enzymes for laundry purposes include, but are not limited to, proteases, cellulases, lipases and peroxidases. Highly preferred for automatic dishwashing are amylases and/or proteases, including both current commercially available types and improved types which, though more and more bleach compatible though successive improvements, have a remaining degree of bleach deactivation susceptibility.

Examples of suitable enzymes include, but are not limited to, hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, keratanases, reductases, oxidases, phenoloxidases, lipoxygenases, ligninases, pullulanases, tannases, pentosanases, malanases, β-glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, and known amylases, or mixtures thereof.

Examples of such suitable enzymes are disclosed in U.S. Patent Nos. 5,705,464, 5,710,115, 5,576,282, 5,728,671 and 5,707,950

The cellulases useful in the present invention include both bacterial or fungal cellulases. Preferably, they will have a pH optimum of between 5 and 12 and a specific activity above 50 CEVU/mg (Cellulose Viscosity Unit). Suitable cellulases are disclosed in U.S. Patent 4,435,307, J61078384 and WO96/02653 which discloses fungal cellulase produced respectively from Humicola insolens, Trichoderma, Thielavia and Sporotrichum. EP 739 982 describes cellulases isolated from novel Bacillus species. Suitable cellulases are also disclosed in GB-A-2.075.028; GB-A-2.095.275; DE-OS-2.247.832 and WO95/26398.

Examples of such cellulases are cellulases produced by a strain of Humicola insolens (Humicola grisea var. thermoidea), particularly the Humicola strain DSM 1800.

Other suitable cellulases are cellulases originated from Humicola insolens having a molecular weight of about 50KDa, an isoelectric point of 5.5 and containing 415 amino acids; and a ~43kD endoglucanase derived from Humicola insolens, DSM 1800, exhibiting cellulase activity; a preferred endoglucanase component has the amino acid sequence disclosed in WO 91/17243. Also suitable cellulases are the EGIII cellulases from Trichoderma longibrachiatum described in WO94/21801 to Genencor. Especially suitable cellulases are the cellulases having color care benefits. Examples of such cellulases are cellulases described in European patent application No. 91202879.2, filed November 6, 1991 (Novo). Carezyme and Celluzyme (Novo Nordisk A/S) are especially useful. See also WO91/17244 and WO91/21801. Other suitable cellulases for fabric care and/or cleaning properties are described in WO96/34092, WO96/17994 and WO95/24471.

Cellulases, when present, are normally incorporated in the cleaning composition at levels from 0.0001% to 2% of pure enzyme by weight of the cleaning composition.

Peroxidase enzymes are used in combination with oxygen sources, e.g. percarbonate, perborate, persulfate, hydrogen peroxide, etc and with a phenolic substrate as bleach enhancing molecule. They are used for "solution bleaching", i.e. to prevent transfer of dyes or pigments removed from substrates during wash operations to other substrates in the wash solution. Peroxidase enzymes are known in the art, and include, for example, horseradish peroxidase, ligninase and haloperoxidase such as chloro- and bromoperoxidase. Suitable peroxidases and peroxidase-containing detergent compositions are disclosed, for example, in U.S. Patent Nos. 5,705,464, 5,710,115, 5,576,282, 5,728,671 and 5,707,950, PCT International Application WO 89/099813, WO89/09813 and in European Patent application EP No. 91202882.6, filed on November 6, 1991 and EP No. 96870013.8, filed February 20, 1996. Also suitable is the laccase enzyme.

Enhancers are generally comprised at a level of from 0.1% to 5% by weight of total composition. Preferred enhancers are substitued phenthiazine and phenoxasine 10-Phenothiazinepropionicacid (PPT), 10-ethylphenothiazine-4-carboxylic acid (EPC), 10-phenoxazinepropionic acid (POP) and 10-methylphenoxazine (described in WO 94/12621) and substitued syringates (C3-C5 substitued alkyl syringates) and phenols. Sodium percarbonate or perborate are preferred sources of hydrogen peroxide.

Said peroxidases are normally incorporated in the cleaning composition at levels from 0.0001% to 2% of pure enzyme by weight of the cleaning composition.

Enzymatic systems may be used as bleaching agents. The hydrogen peroxide may also be present by adding an enzymatic system (i.e. an enzyme and a substrate therefore) which is capable of generating hydrogen peroxide at the beginning or during the washing and/or rinsing process. Such enzymatic systems are disclosed in EP Patent Application 91202655.6 filed October 9, 1991.

Other preferred enzymes that can be included in the cleaning compositions of the present invention include lipases. Suitable lipase enzymes for detergent usage include those produced by microorganisms of the Pseudomonas group, such as Pseudomonas stutzeri ATCC 19.154, as disclosed in British Patent 1,372,034. Suitable lipases include those which show a positive immunological cross-reaction with the antibody of the lipase, produced by the microorganism Pseudomonas fluorescent IAM 1057. This lipase is available from Amano Pharmaceutical Co. Ltd., Nagoya, Japan, under the trade name Lipase P "Amano," hereinafter referred to as "Amano-P". Other suitable commercial lipases include Amano-CES, lipases ex Chromobacter viscosum, e.g. Chromobacter viscosum var. lipolyticum NRRLB 3673 from Toyo Jozo Co., Tagata, Japan; Chromobacter viscosum lipases from U.S. Biochemical Corp., U.S.A. and Disoynth Co., The Netherlands, and lipases ex Pseudomonas gladioli. Especially suitable lipases are lipases such as M1 LipaseR and LipomaxR (Gist-Brocades) and LipolaseR and Lipolase UltraR(Novo) which have found to be very effective when used in combination with the compositions of the present invention. Also suitable are the lipolytic enzymes described in EP 258 068, WO 92/05249 and WO 95/22615 by Novo Nordisk and in WO 94/03578, WO 95/35381 and WO 96/00292 by Unilever.

Also suitable are cutinases [EC 3.1.1.50] which can be considered as a special kind of lipase, namely lipases which do not require interfacial activation. Addition of cutinases to cleaning compositions have been described in e.g. WO-A-88/09367 (Genencor); WO 90/09446 (Plant Genetic System) and WO 94/14963 and WO 94/14964 (Unilever).

Lipases and/or cutinases, when present, are normally incorporated in the cleaning composition at levels from 0.0001% to 2% of pure enzyme by weight of the cleaning composition.

In addition to the above referenced lipases, phospholipases may be incorporated into the cleaning compositions of the present invention. Nonlimiting examples of suitable phospholipases included: EC 3.1.1.32 Phospholipase A1; EC 3.1.1.4 Phospholipase A2; EC 3.1.1.5 Lysopholipase; EC 3.1.4.3 Phospholipase C; EC 3.1.4.4. Phospholipase D. Commercially available phospholipases include LECITASE® from Novo Nordisk A/S of Denmark and Phospholipase A2 from Sigma. When phospolipases are included in the compositions of the present invention, it is preferred that amylases are also included. Without desiring to be bound by theory, it is believed that the combined action of the phospholipase and amylase provide substantive stain removal, especially on greasy/oily, starchy and highly colored stains and soils. Preferably, the phospholipase and amylase, when present, are incorporated into the compositions of the present invention at a pure enzyme weight ratio between 4500:1 and 1:5, more preferably between 50:1 and 1:1.

Suitable proteases are the subtilisins which are obtained from particular strains of B. subtilis and B. licheniformis (subtilisin BPN and BPN'). One suitable protease is obtained from a strain of Bacillus, having maximum activity throughout the pH range of 8-12, developed and sold as ESPERASE® by Novo Industries A/S of Denmark, hereinafter "Novo". The preparation of this enzyme and analogous enzymes is described in GB 1,243,784 to Novo. Proteolytic enzymes also encompass modified bacterial serine proteases, such as those described in European Patent Application Serial Number 87 303761.8, filed April 28, 1987 (particularly pages 17, 24 and 98), and which is called herein "Protease B", and in European Patent Application 199,404, Venegas, published October 29, 1986, which refers to a modified bacterial serine protealytic enzyme which is called "Protease A" herein. Suitable is the protease called herein "Protease C", which is a variant of an alkaline serine protease from Bacillus in which Lysine replaced arginine at position 27, tyrosine replaced valine at position 104, serine replaced asparagine at position 123, and alanine replaced threonine at position 274. Protease C is described in EP 90915958:4, corresponding to WO 91/06637, Published May 16, 1991. Genetically modified variants, particularly of Protease C, are also included herein.

A preferred protease referred to as "Protease D" is a carbonyl hydrolase as described in U.S. Patent No. 5,677,272, and WO95/10591. Also suitable is a carbonyl hydrolase variant of the protease described in WO95/10591, having an amino acid sequence derived by replacement of a plurality of amino acid residues replaced in the precursor enzyme corresponding to position +210 in combination with one or more of the following residues: +33, +62, +67, +76, +100, +101, +103, +104, +107, +128, +129, +130, +132, +135, +156, +158, +164, +166, +167, +170, +209, +215, +217, +218, and +222, where the numbered position corresponds to naturally-occurring subtilisin from *Bacillus amyloliquefaciens* or to equivalent amino acid residues in other carbonyl hydrolases or subtilisins, such as *Bacillus lentus* subtilisin (co-pending patent application US Serial No. 60/048,550, filed June 04, 1997 and PCT International Application Serial No. PCT/IB98/00853).

Also suitable for the present invention are proteases described in patent applications EP 251 446 and WO 91/06637, protease BLAP® described in WO91/02792 and their variants described in WO 95/23221.

See also a high pH protease from Bacillus sp. NCIMB 40338 described in WO 93/18140 A to Novo. Enzymatic detergents comprising protease, one or more other enzymes, and a reversible protease inhibitor are described in WO 92/03529 A to Novo. When desired, a protease having decreased adsorption and increased hydrolysis is available as described in WO 95/07791 to Procter & Gamble. A recombinant trypsin-like protease

for detergents suitable herein is described in WO 94/25583 to Novo. Other suitable proteases are described in EP 516 200 by Unilever.

Particularly useful proteases are described in PCT publications: WO 95/30010; WO 95/30011; and WO 95/29979. Suitable proteases are commercially available as ESPERASE[®], ALCALASE[®], DURAZYM[®], SAVINASE[®], EVERLASE[®] and KANNASE[®] all from Novo Nordisk A/S of Denmark, and as MAXATASE[®], MAXACAL[®], PROPERASE[®] and MAXAPEM[®] all from Genencor International (formerly Gist-Brocades of The Netherlands).

Such proteolytic enzymes, when present, are incorporated in the cleaning compositions of the present invention a level of from 0.0001% to 2%, preferably from 0.001% to 0.2%, more preferably from 0.005% to 0.1% pure enzyme by weight of the composition.

Amylases (α and/or β) can be included for removal of carbohydrate-based stains. WO94/02597 describes cleaning compositions which incorporate mutant amylases. See also WO95/10603. Other amylases known for use in cleaning compositions include both α - and β-amylases. α-Amylases are known in the art and include those disclosed in US Pat. no. 5,003,257; EP 252,666; WO/91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent specification no. 1,296,839 (Novo). Other suitable amylases are stability-enhanced amylases described in WO94/18314 and WO96/05295, Genencor, and amylase variants having additional modification in the immediate parent available from Novo Nordisk A/S, disclosed in WO 95/10603. Also suitable are amylases described in EP 277 216.

Examples of commercial α-amylases products are Purafect Ox Am[®] from Genencor and Termamyl[®], Ban[®], Fungamyl[®] and Duramyl[®], all available from Novo Nordisk A/S Denmark. WO95/26397 describes other suitable amylases: α-amylases characterised by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by the Phadebas[®] α-amylase activity assay. Suitable are variants of the above enzymes, described in WO96/23873 (Novo Nordisk). Other amylolytic enzymes with improved properties with respect to the activity level and the combination of thermostability and a higher activity level are described in WO95/35382.

Such amylolytic enzymes, when present, are incorporated in the cleaning compositions of the present invention a level of from 0.0001% to 2%, preferably from 0.00018% to 0.06%, more preferably from 0.00024% to 0.048% pure enzyme by weight of the composition.

The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic

(psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein / genetic engineering techniques in order to optimize their performance efficiency in the laundry detergent and/or fabric care compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant stability, catalytic activity and the like, of the enzyme variant is tailored to suit the particular cleaning application.

In particular, attention should be focused on amino acids sensitive to oxidation in the case of bleach stability and on surface charges for the surfactant compatibility. The isoelectric point of such enzymes may be modified by the substitution of some charged amino acids, e.g. an increase in isoelectric point may help to improve compatibility with anionic surfactants. The stability of the enzymes may be further enhanced by the creation of e.g. additional salt bridges and enforcing calcium binding sites to increase chelant stability.

These optional detersive enzymes, when present, are normally incorporated in the cleaning composition at levels from 0.0001% to 2% of pure enzyme by weight of the cleaning composition. The enzymes can be added as separate single ingredients (prills, granulates, stabilized liquids, etc... containing one enzyme) or as mixtures of two or more enzymes (e.g. cogranulates).

Other suitable detergent ingredients that can be added are enzyme oxidation scavengers. Examples of such enzyme oxidation scavengers are ethoxylated tetraethylene polyamines.

A range of enzyme materials and means for their incorporation into synthetic detergent compositions is also disclosed in WO 9307263 and WO 9307260 to Genencor International, WO 8908694, and U.S. 3,553,139, January 5, 1971 to McCarty et al. Enzymes are further disclosed in U.S. 4,101,457, and in U.S. 4,507,219. Enzyme materials useful for liquid detergent formulations, and their incorporation into such formulations, are disclosed in U.S. 4,261,868.

Enzyme Stabilizers - Enzymes for use in detergents can be stabilized by various techniques. Enzyme stabilization techniques are disclosed and exemplified in U.S. 3,600,319, EP 199,405 and EP 200,586. Enzyme stabilization systems are also described, for example, in U.S. 3,519,570. A useful Bacillus, sp. AC13 giving proteases, xylanases and cellulases, is described in WO 9401532. The enzymes employed herein can be stabilized by the presence of water-soluble sources of calcium and/or magnesium ions in the finished compositions which provide such ions to the enzymes. Suitable enzyme stabilizers and levels of use are described in U.S. Pat. Nos. 5,705,464, 5,710,115 and 5,576,282.

<u>Builders</u> - The detergent and cleaning compositions described herein preferably comprise one or more detergent builders or builder systems. When present, the compositions will typically comprise at least about 1% builder, preferably from about 5%, more preferably from about 10% to about 80%, preferably to about 50%, more preferably to about 30% by weight, of detergent builder. Lower or higher levels of builder, however, are not meant to be excluded.

Preferred builders for use in the detergent and cleaning compositions, particularly dishwashing compositions, described herein include, but are not limited to, water-soluble builder compounds, (for example polycarboxylates) as described in U.S. Patent Nos. 5,695,679, 5,705,464 and 5,710,115. Other suitable polycarboxylates are disclosed in U.S. Patent Nos. 4,144,226, 3,308,067 and 3,723,322. Preferred polycarboxylates are hydroxycarboxylates containing up to three carboxy groups per molecule, more particularly titrates.

Inorganic or P-containing detergent builders include, but are not limited to, the alkali metal, ammonium and alkanolammonium salts of polyphosphates (exemplified by the tripolyphosphates, pyrophosphates, and glassy polymeric meta-phosphates), phosphonates (see, for example, U.S. Patent Nos. 3,159,581; 3,213,030; 3,422,021; 3,400,148 and 3,422,137), phytic acid, silicates, carbonates (including bicarbonates and sesquicarbonates), sulphates, and aluminosilicates.

However, non-phosphate builders are required in some locales. Importantly, the compositions herein function surprisingly well even in the presence of the so-called "weak" builders (as compared with phosphates) such as citrate, or in the so-called "underbuilt" situation that may occur with zeolite or layered silicate builders.

Suitable silicates include the water-soluble sodium silicates with an SiO₂:Na₂O ratio of from about 1.0 to 2.8, with ratios of from about 1.6 to 2.4 being preferred, and about 2.0 ratio being most preferred. The silicates may be in the form of either the anhydrous salt or a hydrated salt. Sodium silicate with an SiO₂:Na₂O ratio of 2.0 is the most preferred. Silicates, when present, are preferably present in the detergent and cleaning compositions described herein at a level of from about 5% to about 50% by weight of the composition, more preferably from about 10% to about 40% by weight.

Partially soluble or insoluble builder compounds, which are suitable for use in the detergent and cleaning compositions, particularly granular detergent compositions, include, but are not limited to, crystalline layered silicates, preferably crystalline layered sodium silicates (partially water-soluble) as described in U.S. Patent No. 4,664,839, and sodium aluminosilicates (water-insoluble). When present in detergent and cleaning compositions, these builders are typically present at a level of from about 1% to 80% by weight,

preferably from about 10% to 70% by weight, most preferably from about 20% to 60% by weight of the composition.

Crystalline layered sodium silicates having the general formula NaMSi_xO_{2x+1}·yH₂O wherein M is sodium or hydrogen, x is a number from about 1.9 to about 4, preferably from about 2 to about 4, most preferably 2, and y is a number from about 0 to about 20, preferably 0 can be used in the compositions described herein. Crystalline layered sodium silicates of this type are disclosed in EP-A-0164514 and methods for their preparation are disclosed in DE-A-3417649 and DE-A-3742043. The most preferred material is delta-Na₂SiO₅, available from Hoechst AG as NaSKS-6 (commonly abbreviated herein as "SKS-6"). Unlike zeolite builders, the Na SKS-6 silicate builder does not contain aluminum. NaSKS-6 has the delta-Na₂SiO₅ morphology form of layered silicate. SKS-6 is a highly preferred layered silicate for use in the compositions described herein herein, but other such layered silicates, such as those having the general formula NaMSi_xO_{2x+1}·yH₂O wherein M is sodium or hydrogen, x is a number from 1.9 to 4, preferably 2, and y is a number from 0 to 20, preferably 0 can be used in the compositions described herein. Various other layered silicates from Hoechst include NaSKS-5, NaSKS-7 and NaSKS-11, as the alpha, beta and gamma forms. As noted above, the delta-Na₂SiO₅ (NaSKS-6 form) is most preferred for use herein. Other silicates may also be useful such as for example magnesium silicate, which can serve as a crispening agent in granular formulations, as a stabilizing agent for oxygen bleaches, and as a component of suds control systems.

The crystalline layered sodium silicate material is preferably present in granular detergent compositions as a particulate in intimate admixture with a solid, water-soluble ionizable material. The solid, water-soluble ionizable material is preferably selected from organic acids, organic and inorganic acid salts and mixtures thereof.

Aluminosilicate builders are of great importance in most currently marketed heavy duty granular detergent compositions, and can also be a significant builder ingredient in liquid detergent formulations. Aluminosilicate builders have the empirical formula:

$$[M_z(AlO_2)_v] \cdot xH_2O$$

wherein z and y are integers of at least 6, the molar ratio of z to y is in the range from 1.0 to about 0.5, and x is an integer from about 15 to about 264. Preferably, the aluminosilicate builder is an aluminosilicate zeolite having the unit cell formula:

$$Na_z[(AlO_2)_z(SiO_2)_y] \cdot xH_2O$$

wherein z and y are at least 6; the molar ratio of z to y is from 1.0 to 0.5 and x is at least 5, preferably 7.5 to 276, more preferably from 10 to 264. The aluminosilicate builders are preferably in hydrated form and are preferably crystalline, containing from about 10% to about 28%, more preferably from about 18% to about 22% water in bound form.

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These aluminosilicate ion exchange materials can be crystalline or amorphous in structure and can be naturally-occurring aluminosilicates or synthetically derived. A method for producing aluminosilicate ion exchange materials is disclosed in U.S. 3,985,669. Preferred synthetic crystalline aluminosilicate ion exchange materials useful herein are available under the designations Zeolite A, Zeolite B, Zeolite P, Zeolite X, Zeolite MAP and Zeolite HS and mixtures thereof. In an especially preferred embodiment, the crystalline aluminosilicate ion exchange material has the formula:

$$Na_{12}[(AlO_2)_{12}(SiO_2)_{12}] \cdot xH_2O$$

wherein x is from about 20 to about 30, especially about 27. This material is known as Zeolite A. Dehydrated zeolites (x = 0 - 10) may also be used herein. Preferably, the aluminosilicate has a particle size of about 0.1-10 microns in diameter. Zeolite X has the formula:

$$Na_{86}[(AlO_2)_{86}(SiO_2)_{106}] \cdot 276H_2O$$

Citrate builders, e.g., citric acid and soluble salts thereof (particularly sodium salt), are polycarboxylate builders of particular importance for heavy duty liquid detergent formulations due to their availability from renewable resources and their biodegradability. Citrates can also be used in granular compositions, especially in combination with zeolite and/or layered silicate builders. Oxydisuccinates are also especially useful in such compositions and combinations.

Also suitable in the detergent compositions described herein are the 3,3-dicarboxy-4-oxa-1,6-hexanedioates and the related compounds disclosed in U.S. 4,566,984. Useful succinic acid builders include the C₅-C₂₀ alkyl and alkenyl succinic acids and salts thereof. A particularly preferred compound of this type is dodecenylsuccinic acid. Specific examples of succinate builders include: laurylsuccinate, myristylsuccinate, palmitylsuccinate, 2-dodecenylsuccinate (preferred), 2-pentadecenylsuccinate, and the like. Laurylsuccinates are the preferred builders of this group, and are described in European Patent Application 86200690.5/0,200,263, published November 5, 1986.

Fatty acids, e.g., C₁₂-C₁₈ monocarboxylic acids, can also be incorporated into the compositions alone, or in combination with the aforesaid builders, especially citrate and/or the succinate builders, to provide additional builder activity. Such use of fatty acids will generally result in a diminution of sudsing, which should be taken into account by the formulator.

<u>Dispersants</u> - One or more suitable polyalkyleneimine dispersants may be incorporated into the cleaning compositions of the present invention. Examples of such suitable dispersants can be found in European Patent Application Nos. 111,965, 111,984, and 112,592; U.S. Patent Nos. 4,597,898, 4,548,744, and 5,565,145. However, any suitable clay/soil

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dispersent or anti-redepostion agent can be used in the laundry compositions of the present invention.

In addition, polymeric dispersing agents which include polymeric polycarboxylates and polyethylene glycols, are suitable for use in the present invention. Unsaturated monomeric acids that can be polymerized to form suitable polymeric polycarboxylates include acrylic acid, maleic acid (or maleic anhydride), fumaric acid, itaconic acid, aconitic acid, mesaconic acid, citraconic acid and methylenemalonic acid. Particularly suitable polymeric polycarboxylates can be derived from acrylic acid. Such acrylic acid-based polymers which are useful herein are the water-soluble salts of polymerized acrylic acid. The average molecular weight of such polymers in the acid form preferably ranges from about 2,000 to 10,000, more preferably from about 4,000 to 7,000 and most preferably from about 4,000 to 5,000. Water-soluble salts of such acrylic acid polymers can include, for example, the alkali metal, ammonium and substituted ammonium salts. Soluble polymers of this type are known materials. Use of polyacrylates of this type in detergent compositions has been disclosed, for example, in U.S. 3,308,067.

Acrylic/maleic-based copolymers may also be used as a preferred component of the dispersing/anti-redeposition agent. Such materials include the water-soluble salts of copolymers of acrylic acid and maleic acid. The average molecular weight of such copolymers in the acid form preferably ranges from about 2,000 to 100,000, more preferably from about 5,000 to 75,000, most preferably from about 7,000 to 65,000. The ratio of acrylate to maleate segments in such copolymers will generally range from about 30:1 to about 1:1, more preferably from about 10:1 to 2:1. Water-soluble salts of such acrylic acid/maleic acid copolymers can include, for example, the alkali metal, ammonium and substituted ammonium salts. Soluble acrylate/maleate copolymers of this type are known materials which are described in European Patent Application No. 66915, published December 15, 1982, as well as in EP 193,360, published September 3, 1986, which also describes such polymers comprising hydroxypropylacrylate. Still other useful dispersing agents include the maleic/acrylic/vinyl alcohol terpolymers. Such materials are also disclosed in EP 193,360, including, for example, the 45/45/10 terpolymer of acrylic/maleic/vinyl alcohol.

Another polymeric material which can be included is polyethylene glycol (PEG). PEG can exhibit dispersing agent performance as well as act as a clay soil removal-antiredeposition agent. Typical molecular weight ranges for these purposes range from about 500 to about 100,000, preferably from about 1,000 to about 50,000, more preferably from about 1,500 to about 10,000.

Polyaspartate and polyglutamate dispersing agents may also be used, especially in conjunction with zeolite builders. Dispersing agents such as polyaspartate preferably have a molecular weight (avg.) of about 10,000.

Soil Release Agents - The compositions according to the present invention may optionally comprise one or more soil release agents. If utilized, soil release agents will generally comprise from about 0.01%, preferably from about 0.1%, more preferably from about 0.2% to about 10%, preferably to about 5%, more preferably to about 3% by weight, of the composition. Nonlimiting examples of suitable soil release polymers are disclosed in: U.S. Patent Nos. 5,728,671; 5,691,298; 5,599,782; 5,415,807; 5,182,043; 4,956,447; 4,976,879; 4,968,451; 4,925,577; 4,861,512; 4,877,896; 4,771,730; 4,711,730; 4,721,580; 4,000,093; 3,959,230; and 3,893,929; and European Patent Application 0 219 048.

Further suitable soil release agents are described in U.S. Patent Nos. 4,201,824; 4,240,918; 4,525,524; 4,579,681; 4,220,918; and 4,787,989; EP 279,134 A; EP 457,205 A; and DE 2,335,044.

Chelating Agents - The compositions of the present invention herein may also optionally contain a chelating agent which serves to chelate metal ions and metal impurities which would otherwise tend to deactivate the bleaching agent(s). Useful chelating agents can include amino carboxylates, phosphonates, amino phosphonates, polyfunctionally-substituted aromatic chelating agents and mixtures thereof. Further examples of suitable chelating agents and levels of use are described in U.S. Pat. Nos. 5,705,464, 5,710,115, 5,728,671 and 5,576,282.

The compositions herein may also contain water-soluble methyl glycine diacetic acid (MGDA) salts (or acid form) as a chelant or co-builder useful with, for example, insoluble builders such as zeolites, layered silicates and the like.

If utilized, these chelating agents will generally comprise from about 0.1% to about 15%, more preferably from about 0.1% to about 3.0% by weight of the detergent compositions herein.

<u>Suds suppressor</u> - Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Examples of suitable suds suppressors are disclosed in U.S. Patent Nos. 5,707,950 and 5,728,671. These suds suppressors are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

<u>Softening agents</u> - Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the present invention. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400 898 and in U.S. 5,019,292. Organic softening agents include the water insoluble tertiary amines as disclosed in GB-A-1 514 276 and EP-B-011 340 and their combination with mono C12-C14 quaternary

ammonium salts are disclosed in EP-B-026 527 and EP-B-026 528 and di-long-chain amides as disclosed in EP-B-0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP-A-0 299 575 and 0 313 146.

Particularly suitable fabric softening agents are disclosed in U.S. Patent Nos. 5,707,950 and 5,728,673.

Levels of smectite clay are normally in the range from 2% to 20%, more preferably from 5% to 15% by weight, with the material being added as a dry mixed component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or dilong chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as molten liquid on to other solid components of the composition.

Biodegradable quaternary ammonium compounds as described in EP-A-040 562 and EP-A-239 910 have been presented as alternatives to the traditionally used di-long alkyl chain ammonium chlorides and methyl sulfates.

Non-limiting examples of softener-compatible anions for the quaternary ammonium compounds and amine precursors include chloride or methyl sulfate.

Dye transfer inhibition - The detergent compositions of the present invention can also include compounds for inhibiting dye transfer from one fabric to another of solubilized and suspended dyes encountered during fabric laundering and conditioning operations involving colored fabrics.

Polymeric dye transfer inhibiting agents

The detergent compositions according to the present invention can also comprise from 0.001% to 10 %, preferably from 0.01% to 2%, more preferably from 0.05% to 1% by weight of polymeric dye transfer inhibiting agents. Said polymeric dye transfer inhibiting agents are normally incorporated into detergent compositions in order to inhibit the transfer of dyes from colored fabrics onto fabrics washed therewith. These polymers have the ability to complex or adsorb the fugitive dyes washed out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye transfer inhibiting agents are polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinylpyrrolidone polymers, polyvinyloxazolidones and polyvinylimidazoles or mixtures thereof. Examples

of such dye transfer inhibiting agents are disclosed in U.S. Patent Nos. 5,707,950 and 5,707,951.

Additional suitable dye transfer inhibiting agents include, but are not limited to, cross-linked polymers. Cross-linked polymers are polymers whose backbone are interconnected to a certain degree; these links can be of chemical or physical nature, possibly with active groups n the backbone or on branches; cross-linked polymers have been described in the Journal of Polymer Science, volume 22, pages 1035-1039.

In one embodiment, the cross-linked polymers are made in such a way that they form a three-dimensional rigid structure, which can entrap dyes in the pores formed by the three-dimensional structure. In another embodiment, the cross-linked polymers entrap the dyes by swelling. Such cross-linked polymers are described in the co-pending European patent application 94870213.9.

Addition of such polymers also enhances the performance of the enzymes according the invention.

pH and Buffering Variation - Many of the detergent and cleaning compositions described herein will be buffered, i.e., they are relatively resistant to pH drop in the presence of acidic soils. However, other compositions herein may have exceptionally low buffering capacity, or may be substantially unbuffered. Techniques for controlling or varying pH at recommended usage levels more generally include the use of not only buffers, but also additional alkalis, acids, pH-jump systems, dual compartment containers, etc., and are well known to those skilled in the art.

The preferred ADD compositions herein comprise a pH-adjusting component selected from water-soluble alkaline inorganic salts and water-soluble organic or inorganic builders as described in U.S. Patent Nos. 5,705,464 and 5,710,115.

Material Care Agents - The preferred ADD compositions may contain one or more material care agents which are effective as corrosion inhibitors and/or anti-tarnish aids as described in U.S. Patent Nos. 5,705,464, 5,710,115 and 5,646,101.

When present, such protecting materials are preferably incorporated at low levels, e.g., from about 0.01% to about 5% of the ADD composition.

Other Materials - Detersive ingredients or adjuncts optionally included in the instant compositions can include one or more materials for assisting or enhancing cleaning performance, treatment of the substrate to be cleaned, or designed to improve the aesthetics of the compositions. Adjuncts which can also be included in compositions of the present invention, at their conventional art-established levels for use (generally, adjunct materials comprise, in total, from about 30% to about 99.9%, preferably from about 70% to about 95%, by weight of the compositions), include other active ingredients such as non-phosphate builders, color speckles, silvercare, anti-tarnish and/or anti-corrosion agents,

dyes, fillers, germicides, alkalinity sources, hydrotropes, anti-oxidants, perfumes, solubilizing agents, carriers, processing aids, pigments, and pH control agents as described in U.S. Patent Nos. 5,705,464, 5,710,115, 5,698,504, 5,695,679, 5,686,014 and 5,646,101.

Methods of Cleaning - In addition to the methods for cleaning fabrics, dishes and other hard surfaces, and body parts by personal cleansing, described herein, the invention herein also encompasses a laundering pretreatment process for fabrics which have been soiled or stained comprising directly contacting said stains and/or soils with a highly concentrated form of the cleaning composition set forth above prior to washing such fabrics using conventional aqueous washing solutions. Preferably, the cleaning composition remains in contact with the soil/stain for a period of from about 30 seconds to 24 hours prior to washing the pretreated soiled/stained substrate in conventional manner. More preferably, pretreatment times will range from about 1 to 180 minutes.

The following examples are meant to exemplify compositions of the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention.

In all of the following examples Protease¹ means a protease variant comprising substitution of amino acid residues with another naturally occurring amino acid residue at positions corresponding to positions 101G/103A/104I/159D/232V/236H/245R/248D/252K of *Bacillus amyloliquefaciens* subtilisin. Protease¹ can be substituted with any other additional protease variant of the present invention, with substantially similar results in the following examples.

In the cleaning composition examples of the present invention, the Protease¹ enzyme levels are expressed by pure enzyme by weight of the total composition, the other enzyme levels are expressed by raw material by weight of the total composition, and unless otherwise specified, the other ingredients are expressed by weight of the total composition.

Further, in all of the following examples Amylase³ means an amylase variant according to the present invention.

Further, in the following examples some abbreviations known to those of ordinary skill in the art are used, consistent with the disclosure set forth herein.

Examples 1-7
Liquid Hard Surface Cleaning Compositions

Example No.

				Lxamp	16 140.		
Component	1	2	3	4	5	6	7
Protease ¹	0.05	0.05	0.20	0.02	0.03	0.10	0.03
Protease ²	-	_	_	-	•	0.20	0.1
Amylase ³	- (0.002	0.002	0.0005	0.04	0.0008	0.005
Chelant**	-	_		2.90	2.90	.	_
Citrate	-	-	-	-	-	2.90	2.90
LAS	-	1.95	-	1.95	-	1.95	-
AS	2.00	_	2.20	_	2.20	-	2.20
AES	2.00	_	2.20	-	2.20	_	2.20
Amine Oxide	0.40	_	0.50	_	0.50		0.50
Hydrotrope	-	1.30	_	1.30	_	1.30	-
Solvent***	-	6.30	6.30	6.30	6.30	6.30	6.30
Water and Minors				balance	to 100%		

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

In Examples 6 and 7, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

^{**}Na₄ ethylenediamine diacetic acid

^{***}Diethyleneglycol monohexyl ether

^{****}All formulas adjusted to pH 7

Examples 2-7

<u>Dishwashing Composition</u>

Example No. 3 4 7 Component 6 Protease¹ 0.05 0.50 0.02 0.40 0.10 0.03 Protease² 0.40 0.1 Amylase³ 0.002 0.002 0.0005 0.04 0.0008 0.005 TFAA I 0.90 0.90 0.90 0.90 0.90 0.90 **AES** 12.00 12.00 12.00 12.00 12.00 12.00 2-methyl undecanoic acid 4.50 4.50 4.50 4.50 4.50 4.50 4.50 C₁₂ ethoxy (2) carboxylate 4.50 4.50 4.50 C₁₂ alcohol ethoxylate (4) 3.00 3.00 3.00 3.00 3.00 3.00 Amine oxide 3.00 3.00 3.00 3.00 3.00 3.00 2.00 2.00 2.00 2.00 2.00 2.00 Hydrotrope Ethanol 4.00 4.00 4.00 4.00 4.00 4.00 Mg^{++} (as $MgCl_2$) 0.20 0.20 0.20 0.20 0.20 0.20 Ca⁺⁺ (as CaCl₂) 0.40 0.40 0.40 0.40 0.40 0.40 Water and Minors**** balance to 100%

**** Product pH is adjusted to 7.

In Examples 6 and 7, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

Example 8
Dishwashing Compositions (A&B ADW; C Liquid)

		· ·	
Component	A	<u>B</u>	\mathbf{C}
STPP	17.5	-	-
Citrate		15.0	-
Sodium polyacrylate (MW 4500)	0.80	-	-
Acusol 480N	- .	5.10	-
Potassium carbonate	8.30	-	-
Sodium carbonate	-	8.50	-
2.1r K Silicate	3.99	-	-
2.0r Na Silicate	2.00	-	-

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

3.2r Na Silicate	5.18	-	-
Aluminum tristearate	0.10	-	-
Nonionic surfactant	-	2.50	-
NaAE0.6S	-	-	24.70
Glucose amide	-	-	3.09
C10E8	-	•	4.11
Betaine	-	-	2.06
Amine oxide	-	-	2.06
Magnesium as oxide	-	-	0.49
Hydrotrope	-	-	4.47
Sodium hypochlorite as AvCl ₂	1.15	-	-
Amylase ³	0.002	0.03	0.005
Protease ¹	0.01	0.43	0.05
Balance to 100%			

Example 9
Liquid Dishwashing Compositions (especially suitable under Japanese conditions)

Component	A	<u>B</u>
AE1.4S	24.69	24.69
N-cocoyl N-methyl glucamine	3.09	3.09
Amine oxide	2.06	2.06
Betaine	2.06	2.06
Nonionic surfactant	4.11	4.11
Hydrotrope	4.47	4.47
Magnesium	0.49	0.49
Ethanol	7.2	7.2
LemonEase	0.45	0.45
Geraniol/BHT	-	0.60/0.02
Amylase ³	0.03	0.005
Protease ¹	0.01	0.43
Balance to 100%		

Example 10
Granular Automatic Dishwashing Composition

Component	A	В	C
Citric Acid	15.0	_	<u>-</u>
Citrate	4.0	29.0	15.0
	-	29.0	
Acrylate/methacrylate copolymer	6.0	=	6.0
Acrylic acid maleic acid copolymer	-	3.7	-
Dry add carbonate	9.0	-	20.0
Alkali metal silicate	8.5	17.0	9.0
Paraffin	-	0.5	-
Benzotriazole	-	0.3	-
Amylase ³	1.6	1.6	1.6
Protease ¹	0.2	0.1	0.06
Percarbonate (AvO)	1.5	-	-
Perborate monohydrate	-	0.3	1.5
Perborate tetrahydrate	-	0.9	-
Tetraacetylethylene diamine	3.8	4.4	-
Diethylene triamine penta methyl phosphonic acid	0.13	0.13	0.13
(Mg salt)			
Alkyl ethoxy sulphate - 3 times ethoxylated	3.0	-	-
Alkyl ethoxy propoxy nonionic surfactant	-	1.5	-
Suds suppressor	2.0	-	-
Olin SLF18 nonionic surfactant	-	-	2.0
Sulphate	Balance to	100%	

Example 11

Compact high density (0.96Kg/l) dishwashing detergent compositions A to F in accordance with the invention:

Component	Α	В	C	D	E	F
STPP		51.4	51.4			44.3
Citrate	17.05	<u> </u>		49.6	40.2	
Carbonate	17.50	14.0	20.0		8.0	33.6
Bicarbonate	-		-	26.0		
Silicate	14.81	15.0	8.0	-	25.0	3.6
Metasilicate	2.50	4.5	4.5	-		_
PB1	9.74	7.79	7.79		_	_
PB4	-	-		9.6	-	-

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Percarbonate	_	-	_		11.8	4.8
Nonionic	2.00	1.50_	1.50	2.6	1.9	5.9
TAED	2.39	-	-	3.8	-	1.4
HEDP	1.00	-	-	-	-	_
DETPMP	0.65		-		-	-
Mn TACN	-	-		-	0.008	-
PAAC	-	0.008	0.008	-	-	_
Paraffin	0.50	0.38	0.38	0.6	-	-
Protease ¹	0.1	0.06_	0.05	0.03	0.07	0.01
Amylase ³	1.5	1.5	1.5	2.6	2.1	0.8
ВТА	0.30	0.22	0.22	0.3	0.3	0.3
Polycarboxylate	6.0	-			4.2	0.9
Perfume	0.2	0.12	0.12	0.2	0.2	0.2
Sulphate / Water	20.57	1.97	2.97	3.6	4.5	3.9
pH (1% solution)	11.0	11.0	11.3	9.6	10.8	10.9

Example 12

Granular dishwashing detergent compositions examples A to F of bulk density
1.02Kg/L in accordance with the invention:

Component	Α	В	С	D	Е	F
Component	 			 		
STPP	30.00	33.5	27.9	29.62	33.8	22.0
Carbonate	30.50	30.50	30.5	23.00	34.5	45.0
Silicate	7.40	7.50	12.6	13.3	3.2	6.2
Metasilicate	_	4.5				
Percarbonate	-				4.0	
PB1	4.4	4.5	4.3	-	_	
NaDCC	-			2.00	-	0.9
Nonionic	1.0	0.75	1.0	1.90	0.7	0.5
TAED	1.00	-		-	0.9	
PAAC	_	0.004				
Paraffin	0.25	0.25		-	_	
Protease ¹	0.05	0.06	0.025	0.1	0.02	0.07
Amylase ³	0.38	0.64	0.46		0.6	
ВТА	0.15	0.15		-	0.2	
Perfume	0.2	0.2	0.05	0.1	0.2	
Sulphate/water	23.45	16.87	22.26	30.08	21.7	25.4

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pH (1% solution)	10.80	11.3	11.0	10.70	11.5	10.9

Example 13

Tablet detergent composition examples A to H in accordance with the present invention are prepared by compression of a granular dishwashing detergent composition at

a pressure of 13KN/cm² using a standard 12 head rotary press:

a pressure of 13KN/cr	n- usin	g a Stail	uaiu 12 i	icau iota	iry press.			
Component	Α	В	<u>C</u>	D	E	F	G	Н
STPP		48.8	54.7	38.2	-	52.4	56.1	36.0
Citrate	20.0	-	•	-	35.9	-	-	
Carbonate	20.0	5.0	14.0	15.4	8.0	23.0	20.0	28.0
Silicate	15.0	14.8	15.0	12.6	23.4	2.9	4.3	4.2
Protease ¹	0.05	0.09	0.05	0.03	0.06	0.03	0.03	0.1
Amylase ³	1.5	1.5	1.5	0.85	1.9	0.4	2.1	0.3
PB1	14.3	7.8	11.7	12.2		-	6.7	8.5
PB4	_	-	-		22.8	-	3.4	-
Percarbonate	-		-	-		10.4	-	-
Nonionic	1.5	2.0	2.0	2.2	1.0	4.2	4.0	6.5
PAAC	<u> </u>		0.016	0.009		-	-	
MnTACN	<u> </u>		-		0.007	-		-
TAED	2.7	2.4	<u>-</u>		-	2.1	0.7	1.6
HEDP	1.0		<u> - </u>	0.93	<u> </u>	0.4	0.2	
DETPMP	0.7	<u> </u>	-		-	-		
Paraffin	0.4	0.5	0.5	0.55	<u> </u>	_	0.5	
ВТА	0.2	0.3	0.3	0.33	0.3	0.3	0.3	-
Polycarboxylate	4.0		-		4.9	0.6	0.8	-
PEG				<u> </u>	<u> </u>	2.0	-	2.0
Glycerol	-	-	-			0.4		0.5
Perfume	_		-	0.05	0.20	0.2	0.2	0.2
Sulphate / water	17.4	14.7	•	15.74	-	-	-	11.3
weight of tablet	20g	25g	20g	30g	18g	20g	25g	24.0
pH (1% solution)	10.7	10.6	10.7	10.7	10.9	11.2	11.0	10.8

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Example 14

Dimple Tablet Automatic Dishwashing Composition

Diffiple Tablet Automatic Dishwashing Composition									
Component	<u>A</u> (% R.M.)	<u>B</u> (g R.M.)	<u>C</u> (g R.M.)						
Tablet Body									
Sodium Carbonate	15.348	3.500	5.25						
STPP (12% H ₂ O)	46.482	10.600	9.93						
Gran HEDP	0.789	0.180	0.28						
SKS 6	6.578	1.500	2.25						
2 ratio Silicate	7.016	1.600	1.65						
PB1	10.743	2.450	3.68						
Termamyl 2x PCA	0.491	0.112	.17						
Savinase	0.526	0.120	0.18						
Plurafac	3.508	0.800	0.9						
BTA	0.263	0.060	0.09						
PEG	1.140	0.260	-						
PEG 4000	-	-	0.39						
Winog	0.439	0.100	0.15						
Perfume	0.101	0.023	0.01						
Dimple Filling									
Citric Acid	0.987	0.225	0.23						
Bicarbonate	2.600	0.593	0.59						
Sandolan EHRL Dye	0.007	0.0017	0.0017						
PEG 400/4000	0.395	0.090							
PEG 400	-	-	0.02						
PEG 4000	-	-	0.08						
Protease ¹	0.05	0.268	0.27						
Amylase ³	1.412	0.322	0.32						

Granular Fabric Cleaning Composition

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more protease enzymes, preferably from about 0.001% to about 10%, more preferably, from about 0.005% to about 5%, more preferably from 0.01% to about 1% by weight of active protease enzyme of the composition. (See U.S. Patent No. 5,679,630 Examples).

Example 15
Granular Fabric Cleaning Composition

Example No. Component C В D Protease1 0.10 0.20 0.03 0.05 Protease² 0.2 0.15 Amylase³ 0.002 0.0005 0.04 0.005 C₁₃ linear alkyl benzene sulfonate 22.00 22.00 22.00 22.00 Phosphate (as sodium 23.00 23.00 23.00 23.00 tripolyphosphates) Sodium carbonate 23.00 23.00 23.00 23.00 Sodium silicate 14.00 14.00 14.00 14.00 Zeolite 8.20 8.20 8.20 8.20 Chelant (diethylaenetriamine-0.40 0.40 0.40 0.40 pentaacetic acid) Sodium sulfate 5.50 5.50 5.50 5.50 Water_ balance to 100%

In Examples 15 C and D, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

Example 16
Granular Fabric Cleaning Composition

	Example No.					
Component	A	В	С	D		
Protease ¹	0.10	0.20	0.03	0.05		
Protease ²	-	-	0.2	0.1		
Amylase ³	0.005	0.0005	0.04	0.002		
C ₁₂ alkyl benzene sulfonate	12.00	12.00	12.00	12.00		

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

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1	1	6
7		v

2-butyl octanoic acid C ₁₂ -C ₁₄ secondary (2,3) alkyl sulfate,	4.00 5.00	4.00 5.00	4.00 5.00	4.00 5.00
Na salt				
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Fillers, water, minors	balance to 100%			

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

In Examples 16 C and D, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

Example 17
Granular Fabric Cleaning Compositions

Components	Example No.		
	A	<u>B</u>	
Linear alkyl benzene sulphonate	11.4	10.70	
Tallow alkyl sulphate	1.80	2.40	
C ₁₄₋₁₅ alkyl sulphate	3.00	3.10	
C ₁₄₋₁₅ alcohol 7 times ethoxylated	4.00	4.00	
Tallow alcohol 11 times ethoxylated	1.80	1.80	
Dispersant	0.07	0.1	
Silicone fluid	0.80	0.80	
Trisodium citrate	14.00	15.00	
Citric acid	3.00	2.50	
Zeolite	32.50	32.10	
Maleic acid acrylic acid copolymer	5.00	5.00	
Diethylene triamine penta methylene	1.00	0.20	
phosphonic acid			

Protease ¹	0.1	0.01
Lipase	0.36	0.40
Amylase ³	0.30	0.30
Sodium silicate	2.00	2.50
Sodium sulphate	3.50	5.20
Polyvinyl pyrrolidone	0.30	0.50
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.1	0.1
Minors	Up to 100	Up to 100

Example 18
Granular Fabric Cleaning Compositions

	Example No.	
Components	A	<u>B</u>
Sodium linear C ₁₂ alkyl benzene-sulfonate	6.5	8.0
Sodium sulfate	15.0	18.0
Zeolite A	26.0	22.0
Sodium nitrilotriacetate	5.0	5.0
Polyvinyl pyrrolidone	0.5	0.7
Tetraacetylethylene diamine	3.0	3.0
Boric acid	4.0	-
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Protease ¹	0.02	0.05
Amylase ³	0.005	0.002
Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

Example 19 Compact Granular Fabric Cleaning Composition

Components	Weight %
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C25 and C45 alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Protease ¹	0.03
Lipase	0.2
Cellulase	0.2
Amylase ³	0.005
Tetracetylethylene diamine	6.0
Percarbonate	22.0
Ethylene diamine disuccinic acid	0.3
Suds suppressor	3.5
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-	0.25
ylamino) stilbene-2,2'-disulphonate	
Disodium-4,4'-bis (2-sulfostyril) biphenyl	0.05
Water, Perfume and Minors	Up to 100

Example 20 Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C ₁₆ -C ₁₈ alkyl sulfate	1.3
C ₁₄₋₁₅ alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	15.0
Maleic acid acrylic acid copolymer	4.0
Diethylene triamine penta methylene phosphonic acid	0.4
Perborate	15.0
Tetraacetylethylene diamine	5.0
Smectite clay	10.0
Poly (oxy ethylene) (MW 300,000)	0.3
Protease ¹	0.02
Lipase	0.2
Amylase ³	0.3
Cellulase	0.2
Sodium silicate	3.0
Sodium carbonate	10.0
Carboxymethyl cellulose	0.2
Brighteners	0.2
Water perfume and minors	Up to 100

Example 21 Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulfonate	6.92
Tallow alkyl sulfate	2.05
C ₁₄₋₁₅ alcohol 7 times ethoxylated	4.4
C ₁₂₋₁₅ alkyl ethoxy sulfate - 3 times ethoxylated	0.16
Zeolite	20.2
Citrate	5.5
Carbonate	15.4
Silicate	3.0
Maleic acid acrylic acid copolymer	4.0
Carboxymethyl cellulase	0.31
Soil release polymer	0.30
Protease ¹	0.1
Lipase	0.36
Cellulase	0.13
Amylase ³	0.005
Perborate tetrahydrate	11.64
Perborate monohydrate	8.7
Tetraacetylethylene diamine	5.0
Diethylene tramine penta methyl phosphonic acid	0.38
Magnesium sulfate	0.40
Brightener	0.19
Perfume, silicone, suds suppressors	0.85
Minors	Up to 100

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Example 22
Granular Fabric Cleaning Composition

Component	A	В	C
Base Granule Components	44	ACC	
LAS/AS/AES (65/35)	9.95	-	-
LAS/AS/AES (70/30)	-	12.05	7.70
Alumino silicate	14.06	15.74	17.10
Sodium carbonate	11.86	12.74	13.07
Sodium silicate	0.58	0.58	0.58
NaPAA Solids	2.26	2.26	1.47
PEG Solids	1.01	1.12	0.66
Brighteners	0.17	0.17	0.11
DTPA	-	-	0.70
Sulfate	5.46	6.64	4.25
DC-1400 Deaerant	0.02	0.02	0.02
Moisture	3.73	3.98	4.33
Minors	0.31	0.49	0.31
B.O.T. Spray-on			
Nonionic surfactant	0.50	0.50	0.50
Agglomerate Components			
LAS/AS (25/75)	11.70	9.60	10.47
Alumino silicate	13.73	11.26	12.28
Carbonate	8.11	6.66	7.26
PEG 4000	0.59	0.48	0.52
Moisture/Minors	4.88	4.00	4.36
Functional Additives	•		
Sodium carbonate	7.37	6.98	7.45
Perborate	1.03	1.03	2.56
AC Base Coating	-	1.00	-
NOBS	-	-	2.40
Soil release polymer	0.41	0.41	0.31
Cellulase	0.33	0.33	0.24
Protease ¹	0.1	0.05	0.15
Amylase ³	0.002	0.005	0.04
AE-Flake	0.40	0.40	0.29
Liquid Spray-on			
Perfume	0.42	0.42	0.42

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 Noionic spray-on
 1.00
 1.00
 0.50

 Minors
 Up to 100

Example 23
Granular Fabric Cleaning Composition

	A	В
Surfactant		
- Na LAS	6.40	-
-KLAS	-	9.90
- AS/AE3S	6.40	4.39
- TAS	0.08	0.11
- C24AE5	3.48	-
- Genagen	-	1.88
- N-cocoyl N-methyl	1.14	2.82
glucamine (lin)		
- C ₈₋₁₀ dimethyl	1.00	1.40
hydroxyethyl		
ammonium chloride		
Builder		
- Zeolite	20.59	13.39
- SKS-6	10.84	10.78
- Citric Acid	2.00	-
Buffer		
- Carbonate	9.60	12.07
- Bicarbonate	2.00	2.00
- Sulphate	2.64	-
- Silicate	0.61	0.16
Polymer		
- Acrylic acid/maleic	1.17	1.12
acid copolymer (Na)		
- CMC	0.45	0.24

- Polymer	0.34	0.18
- Hexamethylene-	1.00	1.00
diamine tetra-E24		
ethoxylate,		
diquaternized with		
methyl chloride		
Enzyme		
- Protease ¹	0.03	0.03
(% pure enzyme)		
- Cellulase	0.26	0.26
- Amylase ³	0.65	0.73
- Lipase	0.27	0.15
Bleach		
- TAED (100%)	3.85	3.50
- Phenoisulfonate	-	2.75
ester of N-nonanoyl-6-		
aminocaproic acid		
- Percarbonate	16.20	18.30
- HEDP	0.48	0.48
- EDDS	0.30	0.30
Miscellaneous		
- Malic particle		2.20 + bicarb
- Brightener 15/49	0.077/0.014	0.07/0.014
- Zinc phthalocyanine	0.0026	0.0026
sulfonate		
- Polydimethylsiloxane	0.25	0.24
with trimethylsilyl end		
blocking units		
- Soap	-	1.00
- Perfume	0.45	0.55
TOTAL	100	100

Example 24
Granular Fabric Cleaning Composition

	A	В
	%	%
Surfactant		
NaLAS	6.8	0.4
KLAS	-	10.9
FAS	0.9	0.1
AS	0.6	1.5
C25AE3S	0.1	-
AE5	4.2	-
N-Cocoyl-N-Methyl Glucamine	-	1.8
Genagen	-	1.2
C ₈₋₁₀ dimethyl hydroxyethyl	*	1.0
ammonium chloride	alley.	
Builder		
SKS-6	3.3	9.0
Zeolite	17.2	18.9
Citric Acid	1.5	<u>-</u>
Buffer		
Carbonate	21.1	15.0
Sodium Bicarbonate	-	2.6
Sulphate	15.2	5.5
Malic Acid	-	2.9
Silicate	0.1	<u>-</u>
Polymer		
Acrylic acid/maleic acid copolymer	2.2	0.9
(Na)		
Hexamethylene-diamine tetra-E24	0.5	0.7
ethoxylate, diquaternized with		
methyl chloride		
Polymer	0.1	0.1
СМС	0.2	0.1
Enzymes		
Protease ¹ (% pure enzyme)	0.02	0.05
Lipase	0.18	0.14

Amylase ³	0.64	0.73
Cellulase	0.13	0.26
Bleach .		
TAED	2.2	2.5
Phenolsulfonate ester of N-nonanoyl-	-	1.96
6-aminocaproic acid		
Sodium Percarbonate	-	13.1
PB4	15.6	•
EDDS	0.17	0.21
MgSO4	0.35	0.47
HEDP	0.15	0.34
Miscellaneous		
Brightener	0.06	0.04
- Zinc phthalocyanine sulfonate	0.0015	0.0020
- Polydimethylsiloxane with	0.04	0.14
trimethylsilyl end blocking units		
Soap	0.5	0 .7
Perfume	0.35	0.45
Speckle	0.5	0.6

Examples 25

Granular laundry detergent compositions 25 A-C are of particular utility under European machine wash conditions and are prepared in accordance with the invention:

Component	A	В	С	
LAS	7.0	5.61	4.76	
TAS	-	-	1.57	
C45AS	6.0	2.24	3.89	
C25E3S	1.0	0.76	1.18	
C45E7		-	2.0	
C25E3	4.0	5.5	-	

QAS	0.8	2.0	2.0
STPP		-	-
Zeolite A	25.0	19.5	19.5
Citric acid	2.0	2.0	2.0
NaSKS-6	8.0	10.6	10.6
Carbonate I	8.0	10.0	8.6
MA/AA	1.0	2.6	1.6
СМС	0.5	0.4	0.4
PB4	-	12.7	-
Percarbonate	-	-	19.7
TAED		3.1	5.0
Citrate	7.0	-	•
DTPMP	0.25	0.2	0.2
HEDP	0.3	0.3	0.3
QEA 1	0.9	1.2	1.0
Protease ¹	0.02	0.05	0.035
Lipase	0.15	0.25	0.15
Cellulase	0.28	0.28	0.28
Amylase	0.4	0.7	0.3
PVPI/ PVNO	0.4	-	0.1
Photoactivated bleach (ppm)	15 ppm	27 ppm	27 ppm

Brightener 1	0.08	0.19	0.19	
Brightener 2	-	0.04	0.04	
Perfume	0.3	0.3	0.3	
Effervescent granules (malic acid 40%, sodium bicarbonate 40%, sodium carbonate 20%)	15	15		5
Silicone antifoam	0.5	2.4	2.4	
Minors/inerts to 100%				

Example 26

The following formulations are examples of compositions in accordance with the invention, which may be in the form of granules or in the form of a tablet.

Component	26
Base Product	
C45 AS/TAS	3.0
LAS	8.0
C25AE3S	1.0
NaSKS-6	9.0
C25AE5/AE3	5.0
Zeolite A	10.0
SKS-6 (I) (dry add)	2.0
MA/AA	2.0
Citric acid	1.5
EDDS	0.5
HEDP	0.2
PB1	10.0
NACA OBS	2.0
TAED	2.0
Carbonate	8.0
Sulphate	2.0
Amylase ³	0.3
Lipase	0.2
Protease ¹	0.02
Minors (Brightener/SRP1/	0.5
CMC/Photobleach/ MgSO4/	
PVPVI/Suds suppressor/	

Perfume	0.5
1 Cituille	0.5

Example 27

The following granular laundry detergent compositions 27 A-E are of particular utility under Japanese machine wash conditions and are prepared in accord with the invention:

Component	A	В	С	D	E
LAS	23.57	23.57	21.67	21.68	21.68
FAS	4.16	4.16	3.83	3.83	3.83
Nonionic surfactant	3.30	3.30	2.94	3.27	3.27
Bis (hydroxyethyl) methyl alkyl ammonium chloride	0.47	0.47	1.20	1.20	1.20
SKS-6	7.50	7.50	5.17	5.76	5.06
Polyacrylate copolymer (MW 11000) (maleic/acrylate ratio of 4:6)	7.03	7.03	14.36	14.36	14.36
Zeolite	11.90	11.40	10.69	11.34	11.34
Carbonate	14.90	14.82	11.71	11.18	11.18
Silicate	12.00	12.00	12.37	12.38	12.38
Protease ¹	0.016	0.016	0.046	0.046	0.046
Lipase	-	-	0.28	-	-
Amylase ²	-	-	0.62	-	-
Cellulase	-	-	0.48	-	0.70
NOBS	3.75	3.75	2.70	2.70	2.70
PB1	3.53	-	2.60	-	-

Sodium percarbonate	-	4.21	-	3.16	3.16
SRP	0.52	0.52	0.70	0.70	0.70
Brightener	0.31	0.31	0.28	0.28	0.50
AE-coflake	0.17	0.20	0.17	0.17	0.17
Polydimethylsiloxane	-	-	0.68	0.68	0.68
Perfume	0.06	0.06	0.08	-	-
Perfume	-	-	-	0.23	0.23
Hydrophobic precipitated silica	0.30	0.30	0.30	0.30	0.30
PEG4000	0.19	0.19	0.17	0.17	0.17
Minors/inerts to 100%					

Liquid Fabric Cleaning Compositions

Liquid fabric cleaning compositions of the present invention preferably comprise an effective amount of one or more protease enzymes, preferably from about 0.0001% to about 10%, more preferably from about 0.001% to about 1%, and most preferably from about 0.001% to about 0.1% by weight of active protease enzyme of the composition. (See U.S. Patent No. 5,679,630 Examples).

Example 28
Liquid Fabric Cleaning Compositions

Example No. Component В C D E Protease¹ 0.05 0.03 0.30 0.03 0.10 Protease² 0.1 0.20 Amylase³ C₁₂- C₁₄ alkyl sulfate, Na 20.00 20.00 20.00 20.00 20.00 2-Butyl octanoic acid 5.00 5.00 5.00 5.00 5.00 Sodium citrate 1.00 1.00 1.00 1.00 1.00 C₁₀ alcohol ethoxylate (3) 13.00 13.00 13.00 13.00 13.00 Monethanolamine 2.50 2.50 2.50 2.50 2.50 Water/propylene glycol/ethanol (100:1:1) balance to 100%

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

In Examples 28 D and E, any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

Examples 29
Liquid Fabric Cleaning Compositions

	<u>Exampl</u>	e No.
Component C ₁₂₋₁₄ alkenyl succinic acid	<u>A</u> 3.0	<u>B</u> 8.0
Citric acid monohydrate Sodium C ₁₂₋₁₅ alkyl sulphate	10.0 8.0	15.0 8.0
Sodium sulfate of C ₁₂₋₁₅ alcohol 2 times ethoxylated	<u>.</u>	3.0 8.0
C ₁₂₋₁₅ alcohol 7 times ethoxylated C ₁₂₋₁₅ alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta (methylene phosphonic acid)	0.2 1.8	-
Oleic acid Ethanol	4.0	4.0
Propanediol Protease ¹	2.0 0.01	2.0 0.02
Amylase ³	0.005	0.002
Polyvinyl pyrrolidone Suds suppressor	1.0 0.15	2.0 0.15
NaOH	•	pH 7.5
Perborate Phenol sulphonate	0.5 0.1	1 0.2
Peroxidase	0.4	0.1 to 100 %
Waters and minors	w _P	, .

Example 30 Liquid Fabric Cleaning Compositions

	Example No.
Component	<u>28</u>
NaLAS (100%am)	16
Neodol	21.5
Citrate	6.8
EDDS	1.2
Dispersant	1.3
Perborate	12
Phenolsulfonate ester of N-nonanoyl-6-aminocaproic acid	6
Protease ¹ (% pure enzyme)	0.03
Amylase ³	0.40
Carezyme	0.03
Solvent (BPP)	18.5
Polymer	0.1
Carbonate	10
FWA 15	0.2
TiO ₂	0.5
PEG 8000	0.4
Perfume	1.0-1.2
Suds suppressor	0.06
Waters and minors	up to 100%

Examples 31
Liquid Fabric Cleaning Compositions

	Example No.		
Component	Α	B	
DI H₂O	38.63	-	
MEA	0.48	9.0	
NaOH	4.40	1.0	
Pdiol	4.00	10.0	
Citric acid	2.50	2.0	

DTPA	0.50	1.0
FWA Premix (Br 15/MEA/NI 23-9)	0.15	0.15
Na C25AE1.80S	23.50	•
AE3S (H)	-	4.0
C11.8HLAS	3.00	14.0
Neodol	2.00	6.0
EtOH	0.50	2.0
Ca*Formate	0.10	0.1
Borax premix (Borax/MEA/Pdiol/CitricAcid)	2.50	-
C10 APA	1.50	-
TEPA 105	1.20	-
FA C12-18	5.00	-
Neptune LC	0.50	-
Dye	0.0040	0.0015
Cellulase	0.053	0.2
Amylase ³	0.15	0.2
Protease ¹	0.1	0.1
DC 2-3597	0.12	0.2
Rapeseed FA	6.50	4.0
Waters and minors	up to 100 %	6

Example 32
Liquid Fabric Cleaning Composition

Component	<u>30</u>
NaOH	5.50
Pdiol	6.90
Citric acid	1.50
DTPA	1.50
FWA Premix (Br 15/MEA/Nl 23-9)	0.15
AE3S (H)	2.50
LAS (H)	13.0
Neodol	2.00
EtOH	3.50
Ca*Formate	0.10
Boric acid	1.00
Clay	4.00

Amylase ³	0.15
Protease ¹	0.02
Fatty Acid	16.50
Waters and minors	up to 100 %
	1

Example 34
Liquid Fabric Cleaning Composition

The following liquid fabric cleaning composition of particular utility under Japanese machine wash conditions is prepared in accordance with the invention:

Component	34
AE2.5S	15.00
AS	5.50
N-Cocoyl N-methyl glucamine	5.00
Nonionic surfactant	4.50
Citric acid	3.00
Fatty acid	5.00
Base	0.97
Monoethanolamine	5.10
1,2-Propanediol	7.44
EtOH	5.50
HXS	1.90
Boric acid	3.50
Ethoxylated tetraethylene- pentaimine	3.00
SRP	0.30

Protease ¹	0.069
Amylase ³	0.06
Cellulase	0.08
Lipase	0.18
Brightener	0.10
Minors/inerts to 100%	

Example 35
Liquid Fabric Cleaning Composition

The following liquid fabric cleaning composition of particular utility under Japanese machine wash conditions and for fine fabrics is prepared in accordance with the invention:

Component	35
AE2.5S	2.16
AS	3.30
N-Cocoyl N-methyl glucamine	1.10
Nonionic surfactant	10.00
Citric acid	0.40
Fatty acid	0.70
Base	0.85
Monoethanolamine	1.01
1,2-Propanediol	1.92
EtOH	0.24
HXS	2.09

Protease ¹	0.01
Amylase ³	0.06
Minors/inerts to 100%	

Bar Fabric Cleaning Compositions

Bar fabric cleaning compositions of the present invention suitable for handwashing soiled fabrics typically contain an effective amount of one or more protease enzymes, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight active protease enzyme of the composition. (See U.S. Patent No. 5,679,630 Examples).

Example 36
Bar Fabric Cleaning Compositions

Component	Example No.				
	A	В	C	D	
Protease ¹	0.3	-	0.1	0.02	
Protease ²	-	_	0.4	0.1	
Amylase ³	0.01	0.02	0.002	0.005	
C ₁₂ -C ₁₆ alkyl sulfate, Na	20.0	20.0	20.0	20.00	
C ₁₂ -C ₁₄ N-methyl glucamide	5.0	5.0	5.0	5.00	
C ₁₁ -C ₁₃ alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00	
Sodium carbonate	25.0	25.0	25.0	25.00	
Sodium tripolyphosphate	7.0	7.0	7.0	7.00	
Zeolite A (0.110μ)	5.0	5.0	5.0	5.00	
Carboxymethylcellulose	0.2	0.2	0.2	0.20	
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20	
Coconut monethanolamide	5.0	5.0	5.0	5.00	
Brightener, perfume	0.2	0.2	0.2	0.20	
CaSO ₄	1.0	1.0	1.0	1.00	
MgSO ₄	1.0	1.0	1.0	1.00	
Water	4.0	4.0	4.0	4.00	
Filler*	balance to 100%				

^{*}Can be selected from convenient materials such as CaCO3, talc, clay, silicates, and the like.

² Protease other than the Protease¹ including but not limited to the additional proteases useful in the present invention described herein.

In Examples 36 C and D any combination of the protease enzymes useful in the present invention recited herein, among others, are substituted for Protease¹ and Protease², with substantially similar results.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention can be made without departing from the spirit and scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of the invention.

The compositions of the present invention can be suitably prepared by any process chosen by the formulator, non-limiting examples of which are described in U.S. 5,691,297 Nassano et al., issued November 11, 1997; U.S. 5,574,005 Welch et al., issued November 12, 1996; U.S. 5,569,645 Dinniwell et al., issued October 29, 1996; U.S. 5,565,422 Del Greco et al., issued October 15, 1996; U.S. 5,516,448 Capeci et al., issued May 14, 1996; U.S. 5,489,392 Capeci et al., issued February 6, 1996; U.S. 5,486,303 Capeci et al., issued January 23, 1996 all of which are incorporated herein by reference.

In addition to the above examples, the cleaning compositions of the present invention can be formulated into any suitable laundry detergent composition, non-limiting examples of which are described in U.S. 5,679,630 Baeck et al., issued October 21, 1997; U.S. 5,565,145 Watson et al., issued October 15, 1996; U.S. 5,478,489 Fredj et al., issued December 26, 1995; U.S. 5,470,507 Fredj et al., issued November 28, 1995; U.S. 5,466,802 Panandiker et al., issued November 14, 1995; U.S. 5,460,752 Fredj et al., issued October 24, 1995; U.S. 5,458,810 Fredj et al., issued October 17, 1995; U.S. 5,458,809 Fredj et al., issued October 17, 1995; U.S. 5,288,431 Huber et al., issued February 22, 1994 all of which are incorporated herein by reference.

Having described the invention in detail with reference to preferred embodiments and the examples, it will be clear to those skilled in the art that various changes and modifications may be made without departing from the scope of the invention and the invention is not to be considered limited to what is described in the specification.

WHAT IS CLAIMED IS:

- 1. A fabric and/or dishwashing and/or hard surface cleaning composition comprising:
- (a) an effective amount of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin;
- (b) an amylase variant wherein said amylase variant is selected from the group consisting of:
- (i) α -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas[®] α -amylase activity assay and/or;
- (ii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- $(iv) \ \alpha \text{-amylase according to (i) comprising the following amino acid } sequence \ N\text{-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp$

Tyr-Leu-Pro-Asn-Asp (SEQ II \circ 0. 3) or an α -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;

- (v) α -amylase according to (i-iv) wherein the α -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi) α -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii) α -amylase showing positive immunological cross-reactivity with antibodies raised against an α -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent α -amylase, wherein the parent α -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an α-amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an α amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent α-amylase has been deleted; and/or (B) at least one amino acid residue of said parent \(\alpha\)-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α -amylase; said variant having an α -amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or α -amylolytic activity at neutral to relatively high pH values; increased α -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pI) so as to better match the pI value for α -amylase variant to the pH of the medium; and
 - (c) one or more cleaning adjunct materials.
- 2. The cleaning composition according to Claim 1 wherein said protease variant is derived from a *Bacillus* subtilisin, preferably *Bacillus lentus* subtilisin or subtilisin 309.
- 3. The cleaning composition according to Claim 1 wherein said protease variant includes substitutions of the amino acid residues at position 103 and at one or more of the following positions 236 and 245, preferably at positions 103 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 248, 252, 257, 260, 270 and 275 or at positions 103 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 170, 183, 185, 205, 209, 210, 211, 212, 213, 215,

217, 222, 230, 232, 248, 252, 257, 260, 261, 270 and 275, more preferably at positions 103, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 211, 212, 213, 215, 217, 230, 232, 248, 252, 257, 260, 270 and 275.

The cleaning composition according to Claim 1 wherein said protease variant 4. includes a substitution set selected from the group consisting of:

12/102/103/104/159/212/232/236/245/248/252; 12/76/103/104/130/170/185/222/243/245;

12/76/103/104/130/222/245/261;

12/76/103/104/222/245;

12/76/103/104/130/222/245;

61/68/103/104/159/232/236/245/248/252;

62/103/104/159/213/232/236/245/248/252;

62/103/104/109/159/213/232/236/245/248/252; 62/103/104/159/232/236/245/248/252;

62/101/103/104/159/212/213/232/236/245/248/252;

62/103/104/130/159/213/232/236/245/248/252;

68/103/104/159/232/236/245/248/252/270;

68/103/104/159/185/232/236/245/248/252;

68/103/104/159/210/232/236/245/248/252;

68/103/104/159/185/210/232/236/245/248/252; 68/103/104/159/213/232/236/245/248/252;

68/103/104/159/230/232/236/245;

68/76/103/104/159/209/232/236/245;

68/103/104/232/236/245/248/257/275;

68/103/104/213/232/236/245/248/252;

68/103/104/159/232/236/245/248/252;

68/103/104/159/209/232/236/245;

68/76/103/104/159/236;

68/76/103/104/159/236/245;

68/76/103/104/159/232/236/245;

68/103/104/159/232/236/245/252;

68/103/104/159/232/236/245;

68/103/104/159/232/236/245/257;

68/76/103/104/159/211/232/236/245;

68/76/103/104/159/215/232/236/245;

68/103/104/159/210/232/236/245;

68/103/104/159/213/232/236/245/260;

68/76/103/104/159/213/232/236/245/260;

68/103/104/159/236;

68/76/103/104/159/210/232/236/245/260;

68/103/104/159/236/245;

68/103/104/159/183/232/236/245/248/252;

68/76/103/104/159/236/245;

68/103/104/232/236/245/257/275;

68/103/104/159/213/232/236/245;

76/103/222/245;

76/103/104/159/232/236/245;

76/103/104/159/213/232/236/245/260;

76/103/104/159;

76/103/104/131/159/232/236/245/248/252;

76/103/104/222/245;

97/103/104/159/232/236/245/248/252;

98/102/103/104/159/212/232/236/245/248/252; 98/103/104/159/232/236/245/248/252;

101/103/104/159/232/236/245/248/252;

102/103/104/159/232/236/245/248/252;

103/104/159/232/236/245;

103/104/159/232/236/245/248/252;

103/104/159/205/209/232/236/245/257

103/104/159/232/245/248/252;

 103/104/159/205/209/210/232/236/245/257;
 103/104/159/213/232/236/245/248/252;

 103/104/159/217/232/236/245/248/252;
 103/104/130/159/232/236/245/248/252;

 103/104/159/230/236/245;
 103/104/159/236/245;

 103/104/159/248/252/270;
 103/104/131/159/232/236/245/248/252;

 103/104/159/205/209/232/236/245; and
 103/104/159/232/236/245/257.

5. The cleaning composition according to Claim 4 wherein said protease variant includes a substitution set selected from the group consisting of:

12R/76D/103A/104T/130T/222S/245R; 12R/76D/103A/104I/222S/245R;

12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;

12R/76D/103A/104T/130G/222S/245R/261D;

12R/76D/103A/104T/130G/170S/185D/222S/243D/245R;

61E/68A/103A/104I/159D/232V/236H/245R/248D/252K;

62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K;

62D/103A/104I/159D/213R/232V/236H/245R/248D/252K;

62D/103A/104I/159D/232V/236H/245R/248D/252K;

62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K;

62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K;

68A/76D/103A/104I/159D/213R/232V/236H/245R/260A;

68A/103A/104I/159D/236H;

68A/103A/104I/159D/236H/245R;

68A/76D/103A/104I/159D/210I/232V/236H/245R/260A;

68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/209W/232V/236H/245R;

68A/76D/103A/104I/159D/211R/232V/236H/245R;

68A/76D/103A/104I/159D/215R/232V/236H/245R;

68A/103A/104I/159D/213R/232V/236H/245R/260A;

68A/76D/103A/104I/159D/236H;

68A/76D/103A/104I/159D/236H/245R;

68A/76D/103A/104I/159D/232V/236H/245R;

68A/103A/104I/159D/232V/236H/245R/252K;

68A/103A/104I/159D/232V/236H/245R;

68A/103A/104I/159D/232V/236H/245R/257V;

68A/103A/104I/159D/185D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/210L/232V/236H/245R/248D/252K;

68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K;

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68A/103A/104I/159D/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/230V/232V/236H/245R; 68A/76D/103A/104I/159D/209W/232V/236H/245R; 68A/103A/104I/232V/236H/245R/248D/257V/275H; 68A/103A/104I/232V/236H/245R/257V/275H; 68A/103A/104I/213E/232V/236H/245R/248D/252K; 68A/103A/104I/159D/232V/236H/245R/248D/252K; 68A/103A/104I/159D/210I/232V/236H/245R; 68A/103A/104I/159D/210L/232V/236H/245R; 68A/103A/104I/159D/213G/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/248D/252K/270A; 76D/103A/222S/245R: 76D/103A/104I/159D/232V/236H/245R; 76D/103A/104I/159D; 76D/103A/104I/222S/245R; 76D/103A/104I/131V/159D/232V/236H/245R/248D/252K; 76D/103A/104I/159D/213R/232V/236H/245R/260A; 97E/103A/104I/159D/232V/236H/245R/248D/252K; 98L/103A/104I/159D/232V/236H/245R/248D/252K; 98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 101G/103A/104I/159D/232V/236H/245R/248D/252K; 102A/103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/213R/232V/236H/245R/248D/252K; 103A/104I/130G/159D/232V/236H/245R/248D/252K; 103A/104I/159D/230V/236H/245R; 103A/104I/159D/217E/232V/236H/245R/248D/252K; 103A/104I/159D/236H/245R; 103A/104I/159D/248D/252K/270V; 103A/104I/159D/232V/236H/245R; 103A/104I/159D/205I/209W/232V/236H/245R; 103A/104I/159D/232V/236H/245R/257V; 103A/104I/159D/205I/209W/232V/236H/245R/257V; 103A/104I/131V/159D/232V/236H/245R/248D/252K; 103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and 103A/104I/159D/232V/245R/248D/252K.

- 6. The cleaning composition according to Claim 1 wherein said cleaning adjunct materials are selected from the group consisting of surfactants, solvents, buffers, enzymes, soil release agents, clay soil removal agents, dispersing agents, brighteners, suds suppressors, fabric softeners, suds boosters, enzyme stabilizers, builders, other bleaching agents, dyes, perfumes, chelants and mixtures thereof.
- 7. The cleaning composition according to Claim 6 wherein said cleaning adjunct materials comprise at least one detersive surfactant, preferably a branched surfactant, more preferably a mid-chained branched surfactant.
- 8. The cleaning composition according to Claim 7 wherein the cleaning adjunct materials comprise at least about 0.1% surfactant by weight of the composition, said surfactant comprising materials selected from the group consisting of alkyl benzene sulfonates, primary alkyl sulfates, secondary alkyl sulfates, alkyl alkoxy sulfates, alkyl alkoxy sulfates, alkyl alkoxy carboxylates, alkyl polyglycosides and their corresponding sulfated polyglycosides, alpha-sulfonated fatty acid esters, alkyl and alkyl phenol alkoxylates, betaines and sulfobetaines, amine oxides, N-methyl glucamides, nonionic primary alcohol ethoxylates, nonionic primary alcohol mixed ethoxy/propoxy, and mixtures thereof.
- 9. The cleaning composition according to Claim 8 further comprising at least about 5% builder selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof.
- 10. The cleaning composition according to Claim 6 wherein said cleaning adjunct materials comprise at least one detersive enzyme selected from the group consisting of cellulases, lipases, other amylases, phospholipases, other proteases, peroxidases and mixtures thereof.
- 11. The cleaning composition according to Claim 6 wherein said cleaning adjunct materials comprise at least one bleaching agent preferably selected from the group consisting of percarbonates, perborates and mixtures thereof, and optionally further comprising at least one bleach activator preferably selected from the group consisting of benzoyloxybenzenesulphonate (BOBS), nonanoyloxybenzenesulphonate (NOBS), decanoyloxybenzenesulphonate (C₁₀-OBS), octanoyloxybenzenesulphonate (C₈-OBS), perhydrolyzable esters, 4-[N-(nonaoyl) amino hexanoyloxy]-benzene sulfonate sodium salt (NACA-OBS), lauryloxybenzenesulphonate (LOBS or C₁₂-OBS), 10-undecenoyloxybenzenesulfonate (UDOBS or C₁₁-OBS with unsaturation in the 10

position), and decanoyloxybenzoic acid (DOBA) and mixtures thereof, and further optionally comprising a bleach catalyst, preferably 3-(3,4-dihydroisoquinolinium) propane sulfonate.

- 12. The cleaning composition according to Claim 1 wherein said cleaning composition is a fabric cleaning composition, preferably in the form of a liquid, granule, bar, tablet, gel, powder or foam, comprising at least about 5% surfactant and at least about 5% builder by weight of the composition.
- 13. The cleaning composition according to Claim 1 wherein said cleaning composition is a fabric cleaning composition comprising:
 - (a) from about 0.0001% to about 10% by weight of said protease variant;
 - (b) from about 0.0001% to about 0.1% by weight of said amylase variant;
- (c) at least about 5% by weight of a surfactant preferably selected from the group consisting of alkyl benzene sulfonates, primary alkyl sulfates, secondary alkyl sulfates, alkyl alkoxy sulfates, alkyl alkoxy carboxylates, alkyl polyglycosides and their corresponding sulfated polyglycosides, alpha-sulfonated farry acid esters, alkyl and alkyl phenol alkoxylates, betaines and sulfobetaines, amine oxides, N-methyl glucamides, nonionic primary alcohol ethoxylates, nonionic primary alcohol mixed ethoxy/propoxy, and mixtures thereof; and wherein further the builder is selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof; and
- (d) at least about 5% by weight of a builder preferably selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof.
- 14. The cleaning composition according to Claim 25 is in the form of a concentrated granular fabric cleaning composition comprising at least about 15% surfactant.
- 15. A method for cleaning fabric, said method comprising contacting a fabric in need of cleaning with a cleaning composition according to Claims 12 or 13.
- 16. The cleaning composition according to Claim 1 wherein said cleaning composition is a dishwashing composition, preferably in the form of a liquid, granule, powder, gel or tablet, comprising:
 - (a) from about 0.0001% to about 10% by weight of said protease variant;
- (b) from about 0.0001% to about 0.1% by weight of the dishwashing composition of said amylase variant; and
 - (c) from about 0.1% to about 10% by weight of a surfactant.

17. A method for cleaning dishes, said method comprising contacting a dish in need of cleaning with a cleaning composition according to Claim 16.

18. A personal cleansing composition comprising:

- (a) an effective amount of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amyloliquefaciens subtilisin;
- (b) an amylase variant wherein said amylase variant is selected from the group consisting of:
- (i) α -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas® α -amylase activity assay and/or;
- (ii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv) α -amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-

Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an α -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;

- (v) α -amylase according to (i-iv) wherein the α -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi) α -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii) α -amylase showing positive immunological cross-reactivity with antibodies raised against an α -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent α -amylase, wherein the parent α -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an α-amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an α amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent α-amylase has been deleted; and/or (B) at least one amino acid residue of said parent α-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α -amylase; said variant having an α -amylase activity and exhibiting at least one of the following properties relative to said parent α -amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or α -amylolytic activity at neutral to relatively high pH values; increased α -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pl) so as to better match the pI value for α -amylase variant to the pH of the medium; and
 - (c) one or more cleaning adjunct materials.
- 19. The personal cleansing composition according to Claim 18 wherein said personal cleansing composition comprises:
- (a) from about 0.001% to about 5%, preferably from about 0.001% to about 2%, more preferably from about 0.002% to about 0.8% by weight of said protease variant;
- (b) from about 0.0001% to about 0.1% by weight of the personal cleansing composition of said amylase variant; and
- (c) from about 0.1% to about 95% by weight of a surfactant system preferably comprising a surfactant selected from the group consisting of anionic carboxylates, amine oxides, alkyl glucosides, glucose amides, alkyl sulfates, alkyl ether sulfates, acyl isethionates, alkyl sulfosuccinates, alkyl phosphate esters, ethoxylated phosphate esters,

alkyl glyceryl ether sulfonates and mixtures thereof, more preferably comprising a surfactant selected from the group consisting of soaps, acylglutamates, alkyl sarcosinates, lauramine oxides, cocamine oxides, cocamindopropylamine oxides, decylglucosides, lauryl sulfates, laureth sulfates, C₁₂₋₁₈ acyl isethionates and mixtures thereof; and

- (d) optionally, from about 0.05% to about 50% by weight of an enzyme stabilizer.
- 20. The personal cleansing composition according to Claim 19 wherein said surfactant is soap at a level of at least about 2%, preferably at least about 10%, more preferably at least about 25% by weight of the cleaning composition.
- 21. The personal cleansing composition according to Claim 20 wherein the ratio of soap to protease variant is from about 2,000:1 to about 8:1, preferably from about 400:1 to about 40:1.
- 22. A method for personal cleansing, said method comprising contacting a part of the human or lower animal body in need of cleaning with a cleaning composition according to Claim 18.
- 23. A fabric and/or dishwashing and/or hard surface cleaning composition comprising:
- (a) an effective amount of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;
- (b) an amylase variant wherein said amylase variant is selected from the group consisting of:
- (i) α -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas[®] α -amylase activity assay and/or;
- (ii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;

- (iv) α-amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an α-amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;
- (v) α -amylase according to (i-iv) wherein the α -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi) α -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii) α -amylase showing positive immunological cross-reactivity with antibodies raised against an α -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent α -amylase, wherein the parent α -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an α -amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an α amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent \(\alpha\)-amylase has been deleted; and/or (B) at least one amino acid residue of said parent \(\alpha\)-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α-amylase; said variant having an α-amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or α -amylolytic activity at neutral to relatively high pH values; increased α -amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pl) so as to better match the pI value for \alpha-amylase variant to the pH of the medium; and
 - (c) one or more cleaning adjunct materials.
- 24. The cleaning composition according to Claim 23 wherein said protease variant is derived from a *Bacillus* subtilisin, preferably *Bacillus lentus* subtilisin or subtilisin 309.
- 25. The cleaning composition according to Claim 23 wherein said protease variant includes substitutions of the amino acid residues at one or more of the following positions selected from the group consisting of:
- 1) position 62 and at one or more of the following positions 103, 104, 109, 159, 213, 232, 236, 245, 248 and 252;

- 2) position 212 and at one or more of the following positions 12, 98, 102, 103, 104, 159, 232, 236, 245, 248 and 252;
- 3) position 230 and at one or more of the following positions 68, 103, 104, 159, 232, 236 and 245;
- 4) position 232 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 5) position 232 and at one or more of the following positions 103, 104, 236 and 245;
- 6) positions 232 and 103 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 7) positions 232 and 104 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 8) positions 232 and 236 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 9) positions 232 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 10) positions 232, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 76, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 205, 209, 210, 212, 213, 217, 230, 236, 245, 248, 252, 257, 260, 270 and 275;
- 11) position 252 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 12) position 252 and at one or more of the following positions 103, 104, 236 and 245;
- 13) positions 252 and 103 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 14) positions 252 and 104 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;

- 15) positions 252 and 236 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 16) positions 252 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 17) positions 252, 103, 104, 236 and 245 and at one or more of the following positions 12, 61, 62, 68, 97, 98, 101, 102, 103, 104, 109, 130, 131, 159, 183, 185, 210, 212, 213, 217, 232, 236, 245, 248 and 270;
- 18) position 257 and at one or more of the following positions 68, 103, 104, 205, 209, 210, 232, 236, 245 and 275.
- 26. The cleaning composition according to Claim 23 wherein said protease variant includes a substitution set selected from the group consisting of: 12/102/103/104/159/212/232/236/245/248/252; 61/68/103/104/159/232/236/245/248/252; 62/103/104/130/159/213/232/236/245/248/252; 62/103/104/159/213/232/236/245/248/252; 62/103/104/109/159/213/232/236/245/248/252; 62/103/104/159/232/236/245/248/252; 62/101/103/104/159/212/213/232/236/245/248/252; 68/103/104/159/232/236/245/248/252/270; 68/103/104/159/185/232/236/245/248/252; 68/103/104/159/210/232/236/245/248/252; 68/103/104/159/185/210/232/236/245/248/252; 68/103/104/159/213/232/236/245/248/252; 68/103/104/159/230/232/236/245; 68/76/103/104/159/209/232/236/245; 68/103/104/232/236/245/248/257/275; 68/103/104/213/232/236/245/248/252; 68/103/104/159/232/236/245/248/252; 68/103/104/159/209/232/236/245; 68/76/103/104/159/232/236/245; 68/103/104/159/232/236/245/252: 68/103/104/159/232/236/245; 68/103/104/159/232/236/245/257; 68/76/103/104/159/211/232/236/245; 68/76/103/104/159/215/232/236/245; 68/103/104/159/210/232/236/245; 68/103/104/159/213/232/236/245/260; 68/76/103/104/159/213/232/236/245/260; 68/76/103/104/159/210/232/236/245/260; 68/103/104/159/183/232/236/245/248/252; 68/103/104/232/236/245/257/275; 68/103/104/159/213/232/236/245; 76/103/104/159/232/236/245; 76/103/104/159/213/232/236/245/260; 76/103/104/131/159/232/236/245/248/252; 97/103/104/159/232/236/245/248/252; 98/103/104/159/232/236/245/248/252; 98/102/103/104/159/212/232/236/245/248/252; 101/103/104/159/232/236/245/248/252; 102/103/104/159/232/236/245/248/252; 103/104/159/232/236/245; 103/104/159/248/252/270; 103/104/159/232/236/245/248/252; 103/104/159/205/209/232/236/245/257 103/104/159/232/245/248/252:

103/104/159/205/209/210/232/236/245/257;

103/104/159/213/232/236/245/248/252;

103/104/159/217/232/236/245/248/252;

103/104/130/159/232/236/245/248/252;

103/104/131/159/232/236/245/248/252;

103/104/159/205/209/232/236/245; and

103/104/159/232/236/245/257.

27. The cleaning composition according to Claim 26 wherein said protease variant includes a substitution set selected from the group consisting of:

12R/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K;

61E/68A/103A/104I/159D/232V/236H/245R/248D/252K;

62D/103A/104I/109R/159D/213R/232V/236H/245R/248D/252K;

62D/103A/104I/159D/213R/232V/236H/245R/248D/252K;

62D/103A/104I/159D/232V/236H/245R/248D/252K;

62D/103A/104I/130G/159D/213R/232V/236H/245R/248D/252K;

62D/101G/103A/104I/159D/212G/213R/232V/236H/245R/248D/252K;

68A/76D/103A/104I/159D/213R/232V/236H/245R/260A;

68A/76D/103A/104I/159D/210I/232V/236H/245R/260A;

68A/103A/104I/159D/183D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/209W/232V/236H/245R;

68A/76D/103A/104I/159D/211R/232V/236H/245R;

68A/76D/103A/104I/159D/215R/232V/236H/245R;

68A/103A/104I/159D/213R/232V/236H/245R/260A;

68A/76D/103A/104I/159D/232V/236H/245R;

68A/103A/104I/159D/232V/236H/245R/252K;

68A/103A/104I/159D/232V/236H/245R;

68A/103A/104I/159D/232V/236H/245R/257V;

68A/103A/104I/159D/185D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/210L/232V/236H/245R/248D/252K;

68A/103A/104I/159D/185D/210L/232V/236H/245R/248D/252K;

68A/103A/104I/159D/213E/232V/236H/245R/248D/252K;

68A/103A/104I/159D/230V/232V/236H/245R;

68A/76D/103A/104I/159D/209W/232V/236H/245R;

68A/103A/104I/232V/236H/245R/248D/257V/275H;

68A/103A/104I/232V/236H/245R/257V/275H;

68A/103A/104I/213E/232V/236H/245R/248D/252K;

68A/103A/104I/159D/232V/236H/245R/248D/252K;

68A/103A/104I/159D/210I/232V/236H/245R;

68A/103A/104I/159D/210L/232V/236H/245R;

68A/103A/104I/159D/213G/232V/236H/245R; 68A/103A/104I/159D/232V/236H/245R/248D/252K/270A; 76D/103A/104I/159D/232V/236H/245R; 76D/103A/104I/131V/159D/232V/236H/245R/248D/252K; 76D/103A/104I/159D/213R/232V/236H/245R/260A; 97E/103A/104I/159D/232V/236H/245R/248D/252K; 98L/103A/104I/159D/232V/236H/245R/248D/252K; 98L/102A/103A/104I/159D/212G/232V/236H/245R/248D/252K; 101G/103A/104I/159D/232V/236H/245R/248D/252K; 102A/103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/232V/236H/245R/248D/252K; 103A/104I/159D/213R/232V/236H/245R/248D/252K; 103A/104I/130G/159D/232V/236H/245R/248D/252K; 103A/104I/159D/217E/232V/236H/245R/248D/252K; 103A/104I/159D/248D/252K/270V; 103A/104I/159D/232V/236H/245R; 103A/104I/159D/205I/209W/232V/236H/245R; 103A/104I/159D/232V/236H/245R/257V; 103A/104I/159D/205I/209W/232V/236H/245R/257V; 103A/104I/131V/159D/232V/236H/245R/248D/252K; 103A/104I/159D/205I/209W/210I/232V/236H/245R/257V; and 103A/104I/159D/232V/245R/248D/252K.

- 28. The cleaning composition according to Claim 23 wherein said cleaning adjunct materials are selected from the group consisting of surfactants, solvents, buffers, enzymes, soil release agents, clay soil removal agents, dispersing agents, brighteners, suds suppressors, fabric softeners, suds boosters, enzyme stabilizers, builders, other bleaching agents, dyes, perfumes, chelants and mixtures thereof.
- 29. The cleaning composition according to Claim 28 wherein said cleaning adjunct materials comprise at least one detersive surfactant, preferably a branched surfactant, more preferably a mid-chained branched surfactant.
- 30. The cleaning composition according to Claim 28 wherein the cleaning adjunct materials comprise at least about 0.1% surfactant by weight of the composition, said surfactant comprising materials selected from the group consisting of alkyl benzene sulfonates, primary alkyl sulfates, secondary alkyl sulfates, alkyl alkoxy sulfates, alkyl

alkoxy carboxylates, alkyl polyglycosides and their corresponding sulfated polyglycosides, alpha-sulfonated fatty acid esters, alkyl and alkyl phenol alkoxylates, betaines and sulfobetaines, amine oxides, N-methyl glucamides, nonionic primary alcohol ethoxylates, nonionic primary alcohol mixed ethoxy/propoxy, and mixtures thereof.

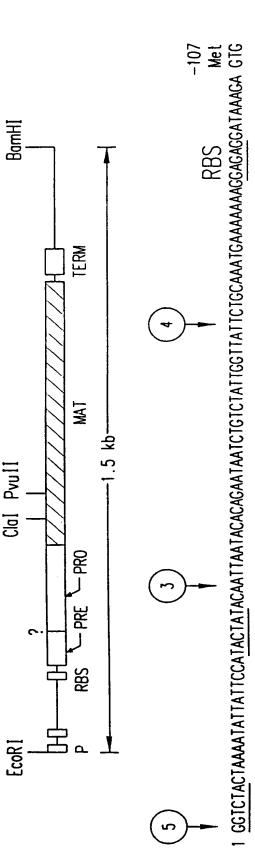
- The cleaning composition according to Claim 30 further comprising at least about 31. 5% builder selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof.
- 32. The cleaning composition according to Claim 28 wherein said cleaning adjunct materials comprise at least one detersive enzyme selected from the group consisting of cellulases, lipases, amylases, phospholipases, other proteases, peroxidases and mixtures thereof.
- 33. The cleaning composition according to Claim 28 wherein said cleaning adjunct materials comprise at least one bleaching agent preferably selected from the group consisting of percarbonates, perborates and mixtures thereof, and optionally further comprising at least one bleach activator preferably selected from the group consisting of benzoyloxybenzenesulphonate (BOBS), nonanoyloxybenzenesulphonate (NOBS), decanoyloxybenzenesulphonate (C10-OBS), octanoyloxybenzenesulphonate (C8-OBS), perhydrolyzable esters, 4-[N-(nonaoyl) amino hexanoyloxy]-benzene sulfonate sodium salt (NACA-OBS), lauryloxybenzenesulphonate (LOBS or C₁₂-OBS), 10undecenoyloxybenzenesulfonate (UDOBS or C₁₁-OBS with unsaturation in the 10 position), and decanoyloxybenzoic acid (DOBA) and mixtures thereof, and further optionally comprising a bleach catalyst, preferably 3-(3,4-dihydroisoquinolinium) propane sulfonate.
- The cleaning composition according to Claim 23 wherein said cleaning 34. composition is a fabric cleaning composition, preferably in the form of a liquid, granule, bar, tablet, gel, powder or foam, comprising at least about 5% surfactant and at least about 5% builder by weight of the composition.
- 35. The cleaning composition according to Claim 23 wherein said cleaning composition is a fabric cleaning composition comprising:
 - from about 0.0001% to about 10% by weight of said protease variant; (a)
- from about 0.0001% to about 0.1% by weight of the fabric cleaning (b) composition of said amylase variant;

- (c) at least about 5% by weight of a surfactant preferably selected from the group consisting of alkyl benzene sulfonates, primary alkyl sulfates, secondary alkyl sulfates, alkyl alkoxy sulfates, alkyl alkoxy carboxylates, alkyl polyglycosides and their corresponding sulfated polyglycosides, alpha-sulfonated farry acid esters, alkyl and alkyl phenol alkoxylates, betaines and sulfobetaines, amine oxides, N-methyl glucamides, nonionic primary alcohol ethoxylates, nonionic primary alcohol mixed ethoxy/propoxy, and mixtures thereof; and wherein further the builder is selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof; and
- (d) at least about 5% by weight of a builder preferably selected from the group consisting of zeolites, polycarboxylates, layered silicates, phosphates, and mixtures thereof.
- 36. The cleaning composition according to Claim 35 is in the form of a concentrated granular fabric cleaning composition comprising at least about 15% surfactant.
- 37. A method for cleaning fabric, said method comprising contacting a fabric in need of cleaning with a cleaning composition according to Claims 34 or 35.
- 38. The cleaning composition according to Claim 23 wherein said cleaning composition is a dishwashing composition, preferably in the form of a liquid, granule, powder, gel or tablet, comprising:
 - (a) from about 0.0001% to about 10% by weight of said protease variant; and
 - (b) from about 0.1% to about 10% by weight of a surfactant.
- 39. A method for cleaning dishes, said method comprising contacting a dish in need of cleaning with a cleaning composition according to Claim 38.
- 40. A personal cleansing composition comprising:
- (a) an effective amount of a protease variant wherein said protease variant includes a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of *Bacillus amyloliquefaciens* subtilisin;
- (b) an amylase variant wherein said amylase variant is selected from the group consisting of:
- (i) α -amylase characterized by having a specific activity at least 25% higher than the specific activity of Termamyl[®] at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by Phadebas[®] α -amylase activity assay and/or;

- (ii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 1 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 1 and/or;
- (iii) α -amylase according to (i) comprising the amino acid sequence shown in SEQ ID No. 2 or an α -amylase being at least 80% homologous with the amino acid sequence shown in SEQ ID No. 2 and/or;
- (iv) α -amylase according to (i) comprising the following amino acid sequence N-terminal: His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp (SEQ ID No. 3) or an α -amylase being at least 80% homologous with the amino acid sequence shown (SEQ ID No. 3) in the N-terminal and/or;
- (v) α -amylase according to (i-iv) wherein the α -amylase is obtainable from an alkalophilic *Bacillus* species and/or;
- (vi) α -amylase according to (v) wherein the amylase is obtainable from any of the strains NCIB 12289, NCIB 12512, NCIB 12513 and DSM 935 and/or;
- (vii) α -amylase showing positive immunological cross-reactivity with antibodies raised against an α -amylase having an amino acid sequence corresponding respectively to SEQ ID No. 1, ID No. 2, or ID No. 3 and/or;
- (viii) variant of a parent α -amylase, wherein the parent α -amylase (1) has one of the amino acid sequences shown in SEQ ID No. 1, ID No. 2, or ID No. 4, respectively, or (2) displays at least 80% homology with one or more of said amino acid sequences, and/or displays immunological cross-reactivity with an antibody raised against an α-amylase having one of said amino acid sequences, and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an \(\alpha\)amylase having one of said amino acid sequences, in which variants: (A) at least one amino acid residue of said parent α-amylase has been deleted; and/or (B) at least one amino acid residue of said parent \alpha-amylase has been replaced by a different amino acid residue; and/or (C) at least one amino acid residue has been inserted relative to said parent α-amylase; said variant having an α-amylase activity and exhibiting at least one of the following properties relative to said parent α-amylase: increased thermostability; increased stability towards oxidation; reduced Ca ion dependency; increased stability and/or α-amylolytic activity at neutral to relatively high pH values; increased α-amylolytic activity at relatively high temperature; and increase or decrease of the isoelectric point (pl) so as to better match the pI value for α-amylase variant to the pH of the medium; and
 - (c) one or more cleaning adjunct materials.
- 41. The personal cleansing composition according to Claim 40 wherein said personal cleansing composition comprises:

- (a) from about 0.001% to about 5%, preferably from about 0.001% to about 2%, more preferably from about 0.002% to about 0.8% by weight of said protease variant;
- (b) from about 0.0001% to about 0.1% by weight of the personal cleansing composition of said amylase variant; and
- (c) from about 0.1% to about 95% by weight of a surfactant system preferably comprising a surfactant selected from the group consisting of anionic carboxylates, amine oxides, alkyl glucosides, glucose amides, alkyl sulfates, alkyl ether sulfates, acyl isethionates, alkyl sulfosuccinates, alkyl phosphate esters, ethoxylated phosphate esters, alkyl glyceryl ether sulfonates and mixtures thereof, more preferably comprising a surfactant selected from the group consisting of soaps, acylglutamates, alkyl sarcosinates, lauramine oxides, cocamine oxides, cocamindopropylamine oxides, decylglucosides, lauryl sulfates, laureth sulfates, C₁₂₋₁₈ acyl isethionates and mixtures thereof; and
- (d) optionally, from about 0.05% to about 50% by weight of an enzyme stabilizer.
- 42. The personal cleansing composition according to Claim 41 wherein said surfactant is soap at a level of at least about 2%, preferably at least about 10%, more preferably at least about 25% by weight of the cleaning composition.
- 43. The personal cleansing composition according to Claim 42 wherein the ratio of soap to protease variant is from about 2,000:1 to about 8:1, preferably from about 400:1 to about 40:1.
- 44. A method for personal cleansing, said method comprising contacting a part of the human or lower animal body in need of cleaning with a cleaning composition according to Claim 40.
- 45. A method for pretreating a fabric in need of cleaning, said method comprising contacting said fabric prior to washing said fabric with an aqueous solution containing a surfactant with a bleaching composition according to Claims 12 or 13.
- 46. A method for pretreating a fabric in need of cleaning, said method comprising contacting said fabric prior to washing said fabric with an aqueous solution containing a surfactant with a bleaching composition according to Claims 34 or 35.





Arg Gly Lys Lys Val Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu Ile Phe Thr Met Ala Phe Gly Ser Thr AGA GGC AAA AAA GTA TGG ATC AGT TTG CTG TTT GCT TTA GCG TTA ATC TTT ACG ATG GCG TTC GGC AGC ACA PRE 66

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-80
Ser Ala Gin Ala Ala Giy Lvs Ser Asn Giv Giu Lys Lys Tvr lie Val Giy Phe Lys Gin Thr Met Ser Thr Met
TCT GCC CAG GCG GCA GGG AAA TCA AAC GGG GAA AAG AAA TAT ATT GTC GGG TTT AAA CAG ACA ATG AGG AGG ATG 174

-50 Ser Ala Ala Lys Lys Lys Asp Val IIe Ser Glu Lys Gly Gly Lys Val Gln Lvs Gln Phe Lvs Tvr Val Asp AGC GCC GCT AAG AAG AAA GAT GTC ATT TCT GAA AAA GGC GGG AAA GTG CAA AAG CAA TTC AAA TAT GTA GAC 249

-30
Alo Ser Alo Thr Leu Asn Glu Lys Alo Vol Lys Glu Leu Lys Lys Asp Pro Ser Vol Alo Tyr Vol Glu Glu Asp 324 GCT TCA GTC ACA TTA AAC GAA AAA GCT GTA AAA GAA TTG AAA AAA GAC CCG AGC GTC GCT TAC GTT GAA GAA GAT

FIG.1A

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GF S¥ S Ser TCT His CAC 10 Bol Alo His Alo Tyr Alo Gln Ser Vol Pro Tyr Gly Vol Ser Gln lle Lys Alo Pro Alo LEu GTA GCA CAT GCG TAC GCG CAG TCC GTG CCT TAC GGC GTA TCA CAA ATT AAA GCC CCT GCT CTG His CAC

30 Thr GLy Ser Asn Val Lys Val Ala Val IIe Asp Ser Gly IIe Asp Ser Ser His Pro Asn Leu Ivs Val ACT GGA TCA AAT GTT AAA GTA GCG GTT ATC GAC AGC GGT ATC GAT TCT TCT CAT CCT GAT TTA AAG GTA 1 3 1

۷۵ا 50 Gly Ala Ser Met Vel Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His Glv Thr His GGA GCC AGC ATG GTT CCT TCT GAA ACA AAT CCT TTC CAA GAC AAC AAC TCT CAC GGA ACT CAC GIY AGC Ala (549 GCA /

Ser Ala 90 Ala Ser Leu Tvr Ala Val Lvs GCA TCA CTT TAC GCT GTA AAA 70 Gly Thr Val Ala Ala Leu Asn Asn Ser IIe Gly Val Leu Gly Val Ala Pro Ser GGC ACA GTT GCG GCT CTT AAT AAC TCA ATC GGT GTA TTA GGC GTT GCG CCA AGC SUBSTITUTE SHEET (RULE 26)

100 Gly Ser Gly Gln Tyr Ser Trp lle lle Asn Gly lle Glu Trp Ala lle Ala Asn Asn GGT TCC GGC CAA TAC AGC TGG ATC ATT AAC GGA ATC GAG TGG GCG ATC GCA AAC AAT Ala Asp GAC Asp A Ala A GCT G Leu CTC

val 11e Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala Ala Leu Lvs Ala Ala Val Asp Lvs Ala Val GTT ATT AAC ATG AGC CTC GGC GGA CCT TCT GGT TCT GCT GCT TTA AAA GCG GCA GTT GAT AAA GCC GTT 120 Asp GAC

Pro CC1 Gly Tvr F Ser Ser Thr Val TCA AGC ACA GTG Thr 160 Ser Gly Ser TCC GGC AGC Ser Thr ACT 150 Val Ala Ala Ala Gly Asn Glu Gly GTT GCG GCA GCC GGT AAC GAA GGC Gly Val Val Val GGC GTC GTA GTC Ser TCC

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CCT Lvs Tvr Pro Ser Val Ile Ala VaL Glv Ala Val Asp Ser Ser Asn Gln Arp Ala Ser Phe Ser Ser Vsl Glv GTA GGA Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Glv Asn Lvs Tvr Gly Ala Tvr Asn 924 AAA TAC CCT TCT GTC ATT GCA GTA GGC GCT GTT GAC AGC AGC AAC CAA AGA GCA TCT TTC TCA AGC 210

GAG CTT GAT GTC ATG GCA CCT GGC GTA TCT ATC CAA AGC ACG CTT CCT GGA AAC AAA TAC GGG GCG TAC AAC

Thr Ser Met Ala Ser Pro His Val Ala Gly Aal Ala Ala Leu Ile Leu Ser Lvs His Pro Asn Trp Thr Asn Thr 1074 ACG TCA ATG GCA TCT CCG CAC GTT GCC GGA GCG GCT GCT TTG ATT CTT TCT AAG CAC CCG AAC TGG ACA AAC ACT

Gln Val Aro Ser Ser Leu Glu Asn Thr Thr Thr Lvs Leu Gly Asp Ser Phe Tyr Tyr Glv Lvs Glv Leu Ile Asn CAA GTC CGC AGC AGT TTA GAA AAC ACC ACT ACA AAA CTT GGT GAT TCT TTG TAC TAT GGA AAA GGG CTG ATC AAC 260 Gln CAA GTC CGC AGC AGT

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Val Gln Ala Ala Ala Gln DC

GTA CAA GCG GCA GCT CAG TAA AAC<u>ATAAAAAACCGGC</u>CTTGGCCCC<u>GCCGGTTTTTTAT</u>TA<u>TTTTT</u>CTTCCTCCGCATGTTCAATCCGCTCC

TERM

1316 ATAATCGACGGATGGCTCCCTCTGAAAATTTTAACGAGAAACGGCGGGTTGACCCGGCTCAGTCCCGTAACGGCCAACTCCTGAAACGTCTCAATCGCCG

1416 CTTCCCGGTTTCCGGTCAGCTCAATGCCATAACGGTCGGCGGCGTTTTCCTGATACCGGGAGACGGCATTCGTAATCGGATC

4/6 CONSERVED RESIDUES IN SUBTILISINS FROM BACILLUS AMYLOLIQUEFACIENS AQSVP.G...APA.H..G . T G S . V K V A V . D . G H P D L . . . G G A S . V P Q D . N . HGTHVAGT . AALNNSIG V L G V A P S A . L Y A V K V L G A . G SG..S.L..G.EWA.N.... V . N . S L G . P S . S A G V . V V A A . G N . G y p . . y A V G A . D . . N . . A S F S . . G . . L D . . A P G V . . Q S T . P G . . Y . . . N G T SMA.PHVAGAAAL...K... W . . . Q . R . . L . N T . . . L G . .

FIG. 2
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Comparison of subtilisin sequences from:

amy lolique faciens а В в в в

subtilis

licheniformis

4 4 4 エエエエ SSS* SSKH 000 999 SSHH 000 8 > > > 4444 >>> \times \times \times \times >>>> zzzo SSAS 9999 >-> 14 -2000 2008 SSAZ THOT AAAA400 AAAA \times \times \times \circ _ _ _ > 20018 R C 0 0 1 8 SSAS 9999 >>>3 <u>a a a a</u> >>>> $S \rightarrow S$ 9000 O A A A

999 zzzz zzoz AAAA4444 AAAA>>>> エエエエ 0000 エエエエ S S S S ZSZZ $z \circ \circ \circ$ 09 99-9 **ட> *** ⊢ d d Z S zz>q \vdash \vdash \prec *تنا تنا تنا تنا 2000 9 4 A B >>>> 50 ▼ 〒 〒 〒 SSSS AAAA 9999 9999 A A > A>>> ¬ \vee Z Z Z 000

0 0 H ΣΣΣΣ zzss zzzz **V**SHZ -1 \forall \forall \forall 3333 تنا تنا تنا تنا 110 G 1 G 1 G 1 G 1 G 1 zzso $H \rightarrow A$ 335V SSSS > > > > 2000 9999 100 G S G S G S 0 1 5 5 ANNA 50 Z 5 >>>> >>>> A> > > > SSSE A < A > ASSSS AVAA >>>> 9999 < < < <

S S S S - SZ Z 9999 $_{\rm S}$ zzzz 0000 VAAV AAAA \forall \forall \forall < > > 0000 SSRR ANAN $>> \succ \vdash$ AAAA140 N D D X N X X S >>>> A > A A4 F 0 0 \times \times \sim \square __ __ __ A A A F $A \vdash \vdash A$ S S S S130 S G T G S G S P A A A 0000 \mathcal{O} \mathcal{O} \mathcal{O} SSSS ΣΣΣ zzzz

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FIG.3B

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SEQUENCE DESCRIPTION : SEQ ID No. 1

His His Asn	Gly Thr Asn	Gly Thr Met	Met Gin Ty	r Phe Glu Trp Tyr
1	5	10	0	15
Leu Pro Asn	Asp Gly As 20	n His Trp As 25	n Arg Leu A	arg Asp Asp Ala Ala 30
Asn Leu Lvs			Vai Tro lie P	ro Pro Ala Trp
35	,	40	- 45	TO THE TIP
Lys Gly Thr	Ser Gin Asn	Asp Val Gly	Tyr Gly Ala	Tyr Asp Leu Tyr
50		55	60	
Asp Leu Gly	Glu Phe As	n Gin Lys Gi	y Thr Val Ar	g Thr Lys Tyr Gly
6 5	70		75	80
Thr Arg Asn		Ala Ala Val	Thr Ser Leu	Lys Asn Asn Gly
	85	9		95
lie Gin Val T	yr Gly Asp	Val Val Met	Asn His Lys	Gly Gly Ala Asp
	00	105		110
Gly Thr Glu	lle Val Asn	Ala Val Glu \	/al Asn Arg	Ser Asn Arg Asn
115		120	125	
Gin Giu Thr	Ser Gly Glu	Tyr Ala lie G	ilu Ala Trp T	hr Lys Phe Asp
130		35	140	
Phe Pro Gly	Arg Gly Asr	Asn His Se	r Ser Phe Ly	rs Trp Arg Trp Tyr
145	150		155	160
His Phe Asp	Gly Thr Asp	Trp Asp Gi	n Ser Arg G	In Leu Gin Asn Lys
	165		170	175
lle Tyr Lys F	Phe Arg Gly	Thr Gly Lys	Ala Trp Asp	Trp Glu Val Asp
	80	185		190
Thr Glu Asn	Gly Asn Ty		u Met Tyr A	la Asp Va! Asp Met
195		20 0	20	
			Arg Asn Trp	Gly Val Trp Tyr
210		15		220
Thr Asn Thr	Leu Asn Le	u Asp Gly Pi	he Arg lie A	sp Ala Val Lys His
225	230		235	240
lie Lys Tyr S	Ser Phe Thr	Arg Asp Trp	Leu Thr His	Val Arg Asn Thr
	245	25		255
Thr Gly Lys	Pro Met Phe	Ala Val Ala	Glu Phe Tr	p Lys Asn Asp Leu
	26 0	265		270

Giy Ala lie	Glu Asn Tyr L	eu Asn Lys	Thr Ser Trp	Asn His Ser	Val
275		280	285	5	
Phe Asp Vi	al Pro Leu His	Tyr Asn Le	u Tyr Asn A	la Ser Asn Se	er Gly
290		295	300		
Gly Tyr Tyr	r Asp Met Arg	Asn lie Leu	Asn Gly Se	er Val Val Gin	Lys
305	310		315	320	
His Pro Thi	His Ala Val 7	nr Phe Val	Asp Asn His	Asp Ser Gin	Pro
	325		_ 330		335
Giy Giu Ala	Leu Glu Ser	Phe Val Gln	Gin Trp Phe	Lys Pro Leu	Ala
	340	345		350	
Tyr Ala Lei	J Val leu Thr	Arg Giu Gin	Giy Tyr Pro	Ser Val Phe	Tyr
355		360	36	5	
Gly Asp Ty	r Tyr Gly lle F	ro Thr His	Gly Val Pro	Ala Met Lys S	Ser
370	375		380		
Lys lie Asp	Pro leu Leu (Sin Ala Arg	Gin Thr Phe	Ala Tyr Gly	Thr
385	390		395	400	
Gin His As	p Tyr Phe Asi	His His As	p lie lie Giy	Trp Thr Arg	Glu
	405	4	10	415	
Gly Asn Si	er Ser His Pro	Asn Ser Gl	y Leu Ala Tr	ar lie Met Ser	Asp
	420	425	•	430	
Gly Pro Gl	y Giy Asn Lys	Trp Met Ty	r Val Gly Ly	s Asn Lys Al	a Gly
4:	35	440	44	5	
Gin Vai Tr	p Arg Asp lie	Thr Gly Asr	Arg Thr Gl	y Thr Val Thr	lie
450	45	i 5	460		
Asn Ala A	sp Gly Trp Gl	y Asn Phe S	er Val Asn	Gly Gly Ser V	al Se
465	470)	475	48	0
Val Trp Va	al Lys Gin				
•	485				

SEQUENCE DESCRIPTION : SEQ ID No. 2

His His Asn (Gly Thr Asn G	ily Thr Met Mi	et Gin Tyr Pi	ne Glu Trp His
1	5	1	_	15
Leu Pro Asn	Asp Gly Asn	His Trp Asn A	rg Leu Arg	Asp Asp Ala Ser
	20	25		30
Asn Leu Arg	Asn Arg Gly	lie Thr Ala lie	Trp lie Pro F	Pro Ala Trp
	35	40 _	45	
Lys Gly Thr	Ser Gin Asn A	sp Val Gly Ty	r Gly Ala Ty	r Asp Leu Tyr
50		55	6 0	
Asp Leu Gly	Glu Phe Asn	Gin Lys Gly T	hr Val Arg T	hr Lys Tyr Gly
65		70	75	
Thr Arg Ser	Gin Leu Glu S	ier Ala lie His	Ala Leu Lys	Asn Asn Gly
.80	85	9	90	9 5
Val Gin Val	Tyr Gly Asp V	'al Val Met As	in His Lys G	ly Gly Ala Asp
	100	105		110
Ala Thr Glu	Asn Val Leu A	Ala Val Glu Va	I Asn Pro A	sn Asn Arg Asn
	115	120		125
Gin Giu lie S	er Gly Asp Ty	yr Thr Ile Glu	Ala Trp Thr	Lys Phe Asp
130		135	140	
Phe Pro Gly	Arg Gly Asn	Thr Tyr Ser A	sp Phe Lys	Trp Arg Trp Tyr
145	1	50	155	
His Phe Asp	Gly Val Asp	Trp Asp Gin S	Ser Arg Gin i	Phe Gln Asn Arg
160	165	•	170	175
He Tyr Lys F	Phe Arg Gly A	sp Gly Lys Al	a Trl Asp Tr	p Glu Val Asp
	180	185		190
Ser Glu Asn	Gly Asn Tyr	Asp Tyr Leu I	Met Tyr Ala	Asp Val Asp Me
	195	200		205
Asp His Pro	Glu Val Val	Asn Glu Leu A	rg Arg Trp (Gly Glu Trp Tyr
210		215	220	
Thr Asn The	r Leu Asn Leu	Asp Gly Phe	Arg lle Asp	Ala Val Lys His
225		230	235	
lie Lys Tyr	Ser Phe Thr A	rg Asp Trp Le	eu Thr His V	al Arg Asn Ala
240	245		250	255
Thr Gly Lys	Glu Met Phe	Ala Val Ala G	Siu Phe Trp L	ys Asn Asp Leu
	260	265		27 0

Gly Ala Leu Glu A	sn Tyr Leu Asn	Lys Thr Asn	Trp Asn H	is Ser Val	
275	21	ВО	285		
Phe Asp Val Pro L	eu His Tyr Asn	Leu Tyr Asn	Ala Ser As	in Ser Gly	
290	295	•	300		
Gly Asn Tyr Asp N	Met Ala Lys Leu	Leu Asn Gi	γ Thr Val Val	al Gin Lys	
305	310	3.	15		
His Pro Met His A	la Val Thr Phe	Val Asp Asn	His Asp Se	r Gln Pro	
320	325	330		335	
Gly Glu Ser Leu G	lu Ser Phe Val	Gin Glu Trp !	Phe Lys Pro	Leu Ala	
34	Ю	345	•		350
Tyr Ala Leu lie Le	u Thr Arg Glu (in Gly Tyr P	ro Ser Val f	Phe Tyr	
355	360)	365		
Gly Asp Tyr Tyr G	Bly lie Pro Thr H	lis Ser Val P	ro Ala Met I	Lys Ala	
370	375		380		
Lys lie Asp Pro lie	Leu Glu Ala A	rg Gln Asn F	Phe Ala Tyr	Gly Thr	
385	390	39 5			
Gln His Asp Tyr P	he Asp His His	Asn lie lie C	Sly Trp Thr	Arg Glu	
400	405	410		415	
Gly Asn Thr Thr	tis Pro Asn Ser	Gly Leu Ala	Thr lie Met	Ser Asp	
42	20	425	430		
Gly Pro Gly Gly G	ilu Lys Trp Met	Tyr Val Gly	Gin Asn Ly	s Ala Gly	
435	44	Ю	445		
Gin Val Trp His A	sp lie Thr Gly	Asn Lys Pro	Gly Thr Val	Thr lie	
450	455		460		
Asn Ala Asp Gly	Trp Ala Asn Pt	ne Ser Val As	sn Giy Giy S	Ser Val Ser	
465	470	4	475		
lie Trp Val Lys A	rg				
480					

SEQUENCE DESCRIPTION : SEQ ID No. 3

His-His-Asn-Gly-Thr-Asn-Gly-Thr-Met-Met-Gln-Tyr-Phe-Glu-Trp-Tyr-Leu-Pro-Asn-Asp

SEQUENCE DESCRIPTION : SEQ ID No. 4

AAPFNGTMMQ	YFEWYLPDDG	TLWTKVANEA	NNLSSLGITA	LWLPPAYKGT
SRSDVGYGVY	DLYDLGEFNO	KGAVRTKYGT	KAQYLQAIQA	
	AHAAGMQVYA			
DVVFDHKGGA	DGTEWVDAVE	VNPSDRNQE	SGTYQIQAWT	KFDFPGRGNT
YSSFKWRWYH	FDGVDWDESR	KLSRIYKFRG	IGKAWDWEVE	
TENGNYDYLM				
YADLDMDHPE	VVTELKSWGK	WYVNTTNIDG	FRLDAVKHIK	FSFFPDWLSD
VRSQTGKPLF	TVGEYWSYD	NKLHNYIMKT	NGTMSLFDAP	LHNKFYTASK
SGGTFDMRTL	MTNTLMKDOP	TLAVTFVDNH	DTEPGOALOS	
	WVDPWFKPLA			
YAFILTROEG	YPCVFYGDYY	GIPOYNIPSL	KSKIDPLLIA	RRDYAYGTQH
DYLDHSDIIG	WTREGVTEKP	GSGLAALITD	GPGGSKWMY	V
GKOHAGKVFY				
DLTGNRSDTV	TINSDGWGEF	KVNGGSVSVW	VPRKTTVSTI	AWSITTRPWT
DEFVRWTEPR	LVAWP			

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(43) International Publication Date 29 April 1999 (29.04.1999)

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(51) International Patent Classification⁶: C11D 3/386, A61K 7/48

(21) International Application Number: PCT/US98/22486

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08/956,323 23 October 1997 (23.10.1997) US 08/956,564 23 October 1997 (23.10.1997) US 08/956,324 23 October 1997 (23.10.1997) US

(71) Applicant (for all designated States except US): THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US).

(72) Inventors; and

- (75) Inventors/Applicants (for US only): GHOSH, Chanchal, Kumar [BD/US]; 7005 Pinemill Drive, West Chester, OH 45069 (US). BAECK, Andre, Cesar [BE/BE]; Putsesteenweg 273, B-2820 Bonheiden (BE). OHTANI, Ryohei [JP/JP]; 7-19, UedaNaka-machi, Nishinomiya, Hyogo, Kobe (JP). BUSCH, Alfred [DE/BE]; Handelstraat 210, B-1840 Londerzeel (BE). SHOWELL, Michael, Stanton [US/US]; 685 Compton Road, Cincinnati, OH 45231 (US).
- (74) Agents: REED, T., David et al.; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217-1087 (US).

- (81) Designated States (national): AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), EE, EE (utility model), ES, FI. FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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 see PCT Gazette No. 35/1999 of 2 September 1999, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MULTIPLY-SUBSTITUTED PROTEASE VARIANT AND AMYLASE VARIANT-CONTAINING CLEANING COMPOSITIONS

(57) Abstract: The present invention relates to cleaning compositions comprising a protease variant. One cleaning composition comprises a protease variant including a substitution of an amino acid residue with another naturally occurring amino acid residue at an amino acid residue position corresponding to position 103 of Bacillus amyloliquefaciens subtilisin in combination with a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 1, 3, 4, 8, 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274 and 275 of Bacillus amyloliquefaciens subtilisin; wherein when said protease variant includes a substitution of amino acid residues at positions corresponding to positions 103 and 76, there is also a substitution of an amino acid residue at one or more amino acid residue positions other than amino acid residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of Bacillus amylolique faciens subtilisin; and one or more cleaning adjunct materials. Another cleaning composition comprises a protease variant including a substitution of an amino acid residue with another naturally occurring amino acid residue at one or more amino acid residue positions corresponding to positions 62, 212, 230, 232, 252 and 257 of Bacillus amyloliquefaciens subtilisin; an amylase variant and one or more cleaning adjunct materials. Methods for using the cleaning compositions are also provided.

WO 99/20723 A3

Internationa. ,illoation No PCT/US 98/22486

		PC1/05 98/	22400
PC 6	CATION OF SUBJECT MATTER C11D3/386 A61K7/48		
	international Patent Classification (IPO) or to both national classifica	ation and IPC	
Minimum do	umentation searched (classification system followed by classification	on sympole)	
IPC 6			
Documentati	on searched other than minimum documentation to the extent that e	such documents are included in the fields seas	ehed
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e pocitió	ENTS CONSIDERED TO BE RELEVANT		
Category *	Chation of document, with indication, where appropriate, of the re	sievant pessages	Relevant to claim No.
Х	WO 97 32961 A (PROCTER & GAMBLE))	1,2, 6-22,45
^	12 September 1997 (1997-09-12)		1-25,
Υ		1	28-46
	the whole document		
Ų	WO 96 23873 A (NOVONORDISK)		1-25,
Y	8 August 1996 (1996-08-08)		28-46
}	cited in the application		
	the whole document		
1	A DE OCCUPA (MONOMODELE)		1-25,
Y	WO 95 26397 A (NOVONORDISK) 5 October 1995 (1995-10-05)		28-46
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X F	urther decuments are listed in the continuation of box C.	X Patent family members are listed	in annex.
* Specia	ostegories of dited documents :	"I later document published after the int or priority date and not in conflict with	emetional filing date
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i lat	er than the priority date claimed	'&' document member of the same pater	
Date of	the actual completion of the international search	Date of making of the international e	_
	26 October 1999	- 8, 11, 199	
Name	and mailing address of the ISA	Authorized officer	
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1	Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,	Neys, P	
l	Fax (+31-70) 340-3016		

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INTERNATIONAL SEARCH REPORT

PCT/US 98/22486

		PC1/US 98/22486
Category *	otion) DOCUMENTS CONSIDERED TO BE RELEVANT Obtation of document, with indication, where appropriate, of the relevant passages	Relevant to plaim No.
		THE BELLY INC.
Y	US 5 679 630 A (BAECK ANDRE ET AL) 21 October 1997 (1997-10-21) cited in the application claims examples table 1	1-25, 28-46
Y	-& WO 95 10591 A (PROCTER & GAMBLE) 20 April 1995 (1995-04-20) the whole document	1-25, 28-46
Y	EP 0 405 901 A (UNILEVER) 2 January 1991 (1991-01-02) claims	1,3-15, 23-25, 28-37,46
	examples	
Y	WO 95 30010 A (PROCTER & GAMBLE) 9 November 1995 (1995-11-09) claims	1,4-25, 28-44,46
Α	examples 7-94 page 68, line 37 -page 73, line 12	26,27
Y	WO 95 30011 A (PROCTER & GAMBLE) 9 November 1995 (1995-11-09) claims	1,4-25, 28-44,46
A	examples 7-94 page 138, line 47 -page 142, line 35	26,27
Y	WO 96 28566 A (PROCTER & GAMBLE) 19 September 1996 (1996-09-19) claims	1,4-25, 28-44,46
A	examples 7-94 page 121, paragraph 2 -page 126, paragraph 3	26,27

REVISED VERSION

(19) World Intellectual Property Organization International Bureau



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(43) International Publication Date 29 April 1999 (29.04.1999)

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- (21) International Application Number:
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- (71) Applicant (for all designated States except US): THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US).
 - (72) Inventors; and
 - (75) Inventors/Applicants (for US only): GHOSH, Chanchal, Kumar [BD/US]; 7005 Pinemill Drive, West Chester, OH 45069 (US). BAECK, Andre, Cesar [BE/BE]; Putsesteenweg 273, B-2820 Bonheiden (BE). OHTANI, Ryohei [JP/JP]; 7-19, UedaNaka-machi, Nishinomiya, Hyogo, Kobe (JP). BUSCH, Alfred [DE/BE]; Handelstraat 210, B-1840 Londerzeel (BE). SHOWELL, Michael, Stanton [US/US]; 685 Compton Road, Cincinnati, OH 45231 (US).
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- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US 98/22486

A CLASSIFICATION OF SUBJECT MATTER IPC 6 C11D3/386 A61 A61K7/48 According to international Patent Cisseffication (IPC) or to both national classification and IPC B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C11D A61K C12N IPC 6 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the International assroh (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to cisim No. Citation of document, with indication, where appropriate, of the relevant passages 1,2, WO 97 32961 A (PROCTER & GAMBLE) Х 6-22,4512 September 1997 (1997-09-12) 1-25. ٧ 28-46 the whole document 1-25. WO 96 23873 A (NOVONORDISK) Y 28-46 8 August 1996 (1996-08-08) cited in the application the whole document 1-25, WO 95 26397 A (NOVONORDISK) Y 5 October 1995 (1995-10-05) 28-45 cited in the application the whole document -/--Potent family members are listed in annex. Further documents are listed in the continuation of box C. X X *T' later document published after the international filling date or priority date and not in conflict with the application but creat to understand the principle or theory underlying the invention * Special categories of olted documents : "A" document defining the general state of the art which is not considered to be of particular relevance. "X" decument of particular relevance; the claimed invention cannot be considered novel or cannot be considered to invalve an inventive step when the document is latent alone "E" earlier document but published on or after the international filling date "L" document which may throw doubte on priority claim(s) of a of another "Y" document of particular relevance; the claimed invention which is cited to establish the publication date citation or other special reason (as specified) cannot be considered to involve an inventive step when the document is combined with one or more other such doou-ments, such combination being obvious to a person eldled in the art. *C* document referring to an oral disclosure, use, exhibition or document published prior to the international filing date but later than the priority date claimed "A" document member of the same patent family Date of mailing of the international search report Date of the actual completion of the international search - B. 11, 1999 26 October 1999 Authorized officer Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijmvlk Tel. (+31-70) 340-2040, Tz. 31 651 epo nl, Fax: (+31-70) 340-3016 Neys, P

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 98/22486

		701/03 90/22486		
C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT Category * Citation of document, with Indication, where appropriate, of the relevant passages Relevant to claim No.				
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Y	US 5 679 630 A (BAECK ANDRE ET AL) 21 October 1997 (1997-10-21) cited in the application claims examples table 1	1-25, 28-46		
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Y	WO 95 30010 A (PROCTER & GAMBLE) 9 November 1995 (1995-11-09) claims examples 7-94	1,4-25, 28-44,46		
A	page 68, line 37 -page 73, line 12	26,27		
Y	WO 95 30011 A (PROCTER & GAMBLE) 9 November 1995 (1995-11-09) claims	1,4-25, 28-44,46		
A	examples 7-94 page 138, line 47 -page 142, line 35	26,27		
Υ	WO 96 28566 A (PROCTER & GAMBLE) 19 September 1996 (1996-09-19) claims	1,4-25, 28-44,46		
A	examples 7-94 page 121, paragraph 2 -page 126, paragraph 3	26,27		